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Toxicity Report No. S.0027395-15

One-generation reproductive toxicity test in Japanese quail (*Coturnix japonica*) exposed orally to 3-nitro-1,2,4-triazol-5-one (NTO), February–August 2015

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Repeated oral exposures to 500 and 1000 mg/kg-day-NTO induced neuromuscular signs and compound-related pre-term mortality in male and female Japanese quail. Birds exhibited convulsions, circling on the floor of the cage, backward arching of the neck (opisthotonos), and alternated between prostrate inactivity and ataxic wing activity. In conjunction with neuromuscular signs, decreased body mass gain occurred in birds as early as one week into exposure. Ultimately, all of the 1000 mg/kg-day birds and all but one of the 500 mg/kg-day birds met euthanasia criteria and were sacrificed. Histopathology could not determine the cause of death in F0 generation birds from the 500 and 1000 mg/kg-day groups.

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Good Laboratory Practice Compliance Statement

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards, except for the following:

1. The test article characterization (purity) was conducted by the manufacturer and it is not known whether the testing was done in compliance with the above regulation.

2. The concentrations of the test article dosing suspensions/solutions for the acute portion of the study were not verified analytically in accordance with Good Laboratory Practice Standards. The accuracy of the data reported is considered sufficient for the purposes of the study.

3. Plasma samples intended to assess immune function were compromised when the -30°C freezer they were being stored in was inadvertently shut off and the samples reached room temperature for an undetermined amount of time.

Study Director:

A Jackovitz

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Biologist
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8/14/2017
Date

TABLE OF CONTENTS

Page

List of Tables

Appendices

TOXICOLOGICAL STUDY NO. S.0027395-15
PROTOCOL NO. 80-14-07-02
ONE-GENERATION REPRODUCTIVE TOXICITY TEST IN JAPANESE QUAIL (COTURNIX JAPONICA) EXPOSED ORALLY TO 3-NITRO-1,2,4-TRIAZOL-5-ONE (NTO)
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1 Summary

1.1 Purpose

The objective of this study was to evaluate the oral toxicity of 3-nitro-1,2,4-triazol-5-one (NTO) as a potential reproductive toxicant in Japanese quail (*Corturnix japonica*).

1.2 Conclusions

Repeated oral exposure to 500 and 1000 mg/kg-day-NTO induced neuromuscular signs and compound-related pre-term mortality in male and female Japanese quail. Following five days of oral exposure, parental generation (F0) birds from the 1000 mg/kg-day group began displaying ataxia. In addition, birds exhibited convulsions, circling on the floor of the cage, and backward arching of the neck (opisthotonos), and alternated between prostrate inactivity and ataxic wing activity beginning 3-4 hours after dosing. In conjunction with neuromuscular signs, decreased body mass gain occurred in birds as early as one week into exposure. After 17 days of exposure, birds from the 500 mg/kg-day group began displaying neuromuscular signs. Ultimately, all of the 1000 mg/kg-day birds and all but one of the 500 mg/kg-day birds met euthanasia criteria and were sacrificed. No NTO-related mortality occurred in the 100 or 20 mg/kg-day groups.

Histopathology could not determine the cause of death in F0 generation birds from the 500 and 1000 mg/kg-day groups. However, vacuolization of the cerebellum and/or the brainstem was observed compared to controls and these changes were present in a dose dependent manner. Neither brain lesions nor convulsions have been seen in previous studies in rodents exposed to NTO.

Mild neuromuscular signs occurred in 10% of first generation (F1) birds from the 100 mg/kg-day group, but not in birds from the 20 mg/kg-day group or control birds in either generation. No other sublethal adverse effects were observed. Tissues from animals exposed to daily doses of 100 and 20 mg/kg-day NTO were generally unaffected. Therefore, mortality was identified as the critical endpoint in this study. A mean BMD of 348 mg/kg-day was calculated for male and female F0 generation quail based on the results of the 5 BMDL models. This corresponded to a $BMDL_{10}$ of 151 mg/kg-day for male and female F0 generation quail.

2 References

See Appendix A for a list of references.

3 Authority

This study was conducted with funding from the Strategic Environmental Research and Development Program (SERDP), Environmental Restoration Project No. ER-2223 (Military

Interdepartmental Purchase Request ((MIPR)) number W74RDV42049178). This toxicology study addresses, in part, the environmental safety and occupational health requirements outlined in Army Regulations (AR) 200-1 and AR 40-5. It was performed as part of a larger effort funded by SERDP entitled "Development of Environmental Health Criteria for Insensitive Munitions".

4 Background

NTO is being investigated as part of a less sensitive (i.e., more resistant to accidental detonation), replacement formulation for traditional explosives such as 2, 4, 6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahyrdo-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). As a potential component of new munitions formulations, NTO must not only meet certain performance criteria, but must also be acceptable from the perspective of human health and the environment. The Army is proactively assembling a spectrum of studies that will ensure accurate environmental assessment of exposure from manufacturing and use of new munition compounds, including NTO. The objective of this study was to assess the toxicity of NTO as a potential reproductive toxicant in Japanese quail (*Corturnix japonica*).

Acute toxicity testing of NTO demonstrated that NTO has low toxicity (LD50 > 5000 mg/kg) in rats and mice [1]. In subacute and subchronic oral studies with rats, limited hematological effects (slight anemia) and liver hyperplasia/hypertrophy occurred at or exceeding 1000 mg/kg-day. The most pronounced effects of oral NTO exposure were decreased testicular and epididymal mass and decreased sperm count [2].

In a reproductive screening study in which NTO was administered at doses between 31.25 and 500 mg/kg-day for two weeks pre-mating, mating and pregnancy rates did not differ significantly between controls and NTO-treated groups [3]. However, sperm counts were reduced by 93% in the 500 mg/kg-day group after four weeks of exposure. Peri-pubertal administration of NTO had no effect on the age or body mass at puberty for male or female rats [4]. In females, there were no marked effects on reproductive endpoints (e.g., estrus cycling, tissue mass); however, in males, there were significant reductions in the mass of the testes and epididymides. In the same study, thyroid hormone levels and histopathology did not differ between NTO treated and control animals.

A battery of *in vivo* and *in vitro* endocrine disruptor screening tests was also conducted. The Hershberger and uterotrophic assays did not demonstrate marked anti-androgenic or estrogenic activity, respectively, for NTO at doses up to 1000 mg/kg-day [5]. Screenings for estrogen receptor binding, androgen receptor binding, estrogen transactivation, aromatase, and steroidogenesis were negative [6]. Therefore, direct testicular toxicity is the most likely mode of action.

An extended one-generation study in rats was also conducted to bridge the gaps between previously conducted studies by evaluating specific life stages not covered by other types of studies and testing for effects that may occur as a result of combined pre- and post-natal exposure [7]. Results support previous work indicating that NTO is a testicular toxicant with male developmental effects that may be secondary to testicular toxicity.

To assess the toxicity of NTO as a potential reproductive toxicant in birds, acute and one-generation reproductive toxicity tests were conducted in Japanese quail. Avian data will be used to derive class-specific Toxicity Reference Values (TRVs) for birds. Avian-specific data also has merit as past regulatory concerns for threatened and endangered bird species, bald eagles, and other protected migratory bird species at installations suggest the need for collection of bird-specific toxicity data.

Table 1 identifies the dates of critical study events.

Table 1. Critical Study Events

Critical Event	Date of Event
Animal Use Protocol Approved	07/03/2014
Study Initiation Date	02/09/2015
Acute Study Initiation	02/09/2015
One-generation Study Initiation	03/09/2015
One-generation Study Necropsy	08/17/2015 – 08/20/2015
Experimental Completion	04/12/2017
Study Completion	08/14/2017

5 Materials and Methods

5.1 Test Substance

3-Nitro-1,2,4-triazol-5-one (CAS #932649; batch 10NTO0-8; lot BAE11C305-009; purity: 100%) was obtained from BAE Systems, Ordnance Systems Kingsport, TN. Dosing suspensions were prepared by weighing the required amount of NTO and suspending it in corn oil. Calculated amounts of NTO for each dosing suspension were wetted with corn oil and ground in a mortar and pestle to facilitate a uniform suspension that would transfer through a gavage tube. For the F0 generation, 4 dosing solutions, 2, 10, 50, and 100 milligrams per milliliter (mg/ml) of NTO, were prepared at the start of the study and replacements were prepared as needed. For the F1 generation, 2 dosing solutions, 2 and 10 mg/ml of NTO, were prepared when the birds hatched. Replacements were prepared as needed.

A one milliliter sample was taken from each prepared dosing suspension and analyzed by the Army Public Health Center (APHC) Laboratory Sciences Directorate via high performance liquid chromatography with ultra violet detection to verify the concentration. Homogeneity was confirmed by taking samples from the top, middle, and bottom of the most concentrated suspension. In conjunction with a previous study, samples were collected from a representative suspension (6 mg/ml) at approximately weekly intervals to determine the stability of the dosing suspension. Results from the stability test indicated that the test compound was stable for at least eight weeks when stored at room temperature. The dosing suspensions were mixed on a stir plate for approximately 30 minutes prior to taking analytical samples, approximately 10 minutes prior to dosing, and continued to be mixed throughout the dosing procedure.

5.2 Animals*

This study was conducted using male and female Japanese quail (*Corturnix japonica*) using an IACUC approved protocol. Eggs were obtained from Georgia Quail Farm Manufacturing Company Inc., Savannah, Georgia. All animals were hatched at APHC. All animals were housed in temperature-, relative humidity-, and light-controlled rooms (16-17 hours of light and 8-7 hours of darkness). Table 2 summarizes the target housing conditions.

Table 2. Housing Conditions

Age (weeks)	Temperature (ºC)	Temperature (ºF)	Relative Humidity (%)
1	35-38	95-100	30-70
2	30-35	86-95	30-70
3-4	23-30	74-86	30-70
> 4	21-27	70-80	30-70

Certified starter feed (Purina®, game bird, Startena® BMD 50, medicated) or breeding feed (Purina®, game bird breeder, Layena® Complete Ration) was available *ad libitum*. Per the protocol, adult birds (i.e., those in adult cages beginning at week 4 of age) were directed to receive a "laying diet." Prompted by observation and documentation of soft and/or broken eggs over the course of a few days, it was discovered that adult birds were inadvertently fed chick food for two weeks (i.e., between weeks 4 and 6). Although consuming the wrong feed impacted egg quality at the time (e.g., strength and thickness), these parameters were measured at the conclusion of the F0 generation (i.e., once birds had been receiving the appropriate diet for 6 weeks).

Chicks were group-housed in a free-range fashion. Filtered tap water was provided *ad libitum*. At 4 weeks of age, quail were moved to adult housing and same-sex pair housed by dosage group. After a few days (once juvenile quail demonstrated competent use of the automated watering system), animals were individually housed. Each animal was uniquely identified by number via cage card and a leg band. Leg bands were color coded according to dosage group.

5.3 Quality Assurance

The APHC Quality Systems and Regulatory Compliance (QSARC) audited critical study phases. Appendix B provides the dates of these audits, the phases audited, and the dates that the results of the inspections were reported to the Study Director (SD) and Management.

5.4 Study Personnel

Appendix C lists the names of individuals contributing to the study performance.

* Animal use procedures were approved by the Army Public Health Center (APHC) Institutional Animal Care and Use Committee. Animal care and use was conducted in accordance with *The Guide for the Care and Use of Laboratory Animals* and all applicable Federal and DOD regulations. The APHC Animal Care and Use Program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

5.5 Avian Acute Oral Toxicity Test

Standard test protocols according to the Organization for Economic Co-Operation and Development (OECD) were used [8]. Acute toxicity testing of NTO demonstrated that NTO has low toxicity (LD_{50} > 5000 mg/kg) in rats and mice, therefore, in compliance with OECD recommendations, only a Limit Dose test was conducted in quail as opposed to more extensive (e.g., higher animal number) sequential tests [6]. Quail were mature in plumage, but not in breeding condition, in accordance with guidelines [8]; therefore, birds were 5 weeks of age at the start of the Limit Dose test. Five animals were tested at the Limit Dose (2000 mg/kg), in addition to a control group consisting of 5 animals. This is the recommended strategy for testing materials that are unlikely to present a significant hazard. Mortality was the primary endpoint in this study and background mortality was presumed to be negligible [8].

Three females and two males were randomly assigned to the control group. Two females and three males were randomly assigned to the Limit Dose test group. Females weighed 148.0 ± 7 grams (g), while males weighed 137.5 ± 6 g. All vehicle control and NTO doses were administered according to the body mass measured on the day of dosing. Oral dosing was performed using a stainless steel 16 gauge x 2 inch gavage needle. After receiving a single dose, all animals were observed for 14 days for signs of toxicity, morbidity, and mortality. Animals were euthanized at the conclusion of the test. If no mortality is observed for 14 days after dosing, it can be concluded with 95% confidence that the LD50 is above the Limit Dose.

During the Avian Acute Oral Toxicity Test, birds were observed continuously during the first 2 hours after dosing for regurgitation and for the onset of clinical signs on at least three evenly spaced additional occasions during the day. Following the initial dosing day, birds were observed for clinical signs at least once daily for 14 days. Observations on each individual included: regurgitation, signs of intoxication and remission, abnormal behavior, mortality, and time to death. Body weights were only collected on the day of dosing. Birds were assessed for morbidity based on loss of righting response, an inability to feed or drink independently, and signs indicative of dehydration or shock (e.g., lethargy, depression, wing droop, ruffled feathers, panting).

5.6 One-Generation Study

A one-generation reproductive toxicity test was performed with NTO. Half the Limit Dose (1000 mg/kg-day) was selected for the high dose. The medium-high dose was set at 500 mg/kg-day, with the medium-low, and low doses set at five-fold intervals (e.g., 100, and 20 mg/kg-day, respectively). All NTO doses and the control were administered based on body mass and volume of suspension at rates of 10 milliliter per kilogram (ml/kg). Dosages were adjusted weekly for changes in body mass for the F0 generation and more frequently between hatch and week 4 for the F1 generation, to ensure that rapidly growing chicks received the appropriate dose. Oral dosing was performed using a stainless steel 16 gauge x 1-2 inch gavage needle.

To produce the F0 generation, 300 eggs were incubated. Between embryonic day (ED) 17 and 19, 264 chicks hatched. At day 10 post-hatching, 260 birds were randomly sorted into 5 treatment groups. At this age, sex of the birds cannot be determined, but the sex ratio was assumed to be 1:1. Treatment began at week 2 post hatch and continued through termination. At week 4, when sex can be determined in quail, as many as 16 birds from each sex and dose group were moved to adult caging. Excess birds were culled. Once birds demonstrated an understanding of the automatic watering system, treatment groups were trimmed down to as many as 12 birds per sex

and dose group, and excess birds were culled. Beginning at week 5, sexual development (including daily egg production and weekly cloaca gland measurements) was assessed. At week 6, immune function was assessed, as described in 5.6.3. At week 7, male copulatory behavior was assessed, as described in 5.6.4. After behavioral assessment and mating to produce eggs for the F1 generation had occurred (i.e., week 12), F0 birds were terminated and necropsied.

To generate the F1 generation, 170 eggs produced by the F0 generation were incubated. Between ED 17 and 19, 127 chicks hatched. Unlike the F0 generation where exposure began at week 2, F1 birds were exposed *in ovo* via material deposition. As such, hatched birds were banded immediately upon hatch to designate in which dose group the parental generation (and thus the F1 generation) belonged. At day 2, oral exposure began and continued until termination. At week 4 (when sex could be determined) up to 18 birds/sex/dose were moved to adult caging. To improve the statistical power of the study (unlike with the F0 generation) sample size was not reduced after the quail demonstrated competent use of the automatic watering system. Beginning at week 5, sexual development (including daily egg production and weekly cloaca gland measurements) was assessed. At week 6, immune function was assessed. At week 7, male copulatory behavior was assessed. After behavioral assessment and mating to produce eggs to incubate to determine fertility (i.e., week 10), F1 birds were terminated and necropsied.

5.6.1 Clinical Observations and Body Mass

Throughout the one generation study, birds were removed from their home cages and observed daily by study personnel in conjunction with dosing. The birds were assessed for morbidity using the same criteria as the Avian Acute Oral Toxicity Test. F0 quail body weights were taken at the start of test compound administration, at least weekly thereafter, and at termination. F1 quail body weights were taken daily between hatch and week 4, at least weekly thereafter, and at termination.

5.6.2 Assessment of Sexual Development

Beginning at week 5, males were observed daily for reproductive maturity, which is determined by the presence or absence of foam dispensed from the cloaca gland. Each immature male bird's foam production was observed daily until it was reproductively mature. Male cloaca glands were measured weekly beginning at week 6. In females, reproductive maturity was determined by the presence of the first egg. Egg production was monitored daily and classified as hard, soft, or broken.

5.6.3 Assessment of Immune Function

Humoral response as an indicator of immunotoxicity was evaluated via a foreign red blood cell (RBC) challenge. Each animal received one 0.1 ml injection of a 5% pig RBC suspension in Phosphate Buffer Solution (PBS). Injections were performed intravenously (IV). For IV injections, a referenced safe maximum volume is 5 ml/kg body weight. IV injections were administered into the jugular vein found along the neck using a 1 ml syringe fitted with a 25 gauge needle. Immunotoxicity testing was done at week 6 in both generations.

Ten days post injection, up to 1 ml of blood was collected from the jugular vein of unanesthetized animals. Blood was placed in Sarstedt lithium heparin microtubes and refrigerated overnight. The next day, plasma was isolated by centrifugation (approximately 5 minutes at 2570 x g). Plasma was stored in 1 ml cryovials in a -30ºC freezer until analysis.

To measure the RBC antibody response, a hemaglutinnation assay was performed in which plasma was thawed, serially diluted in PBS, and re-exposed to the foreign antigen. To simulate re-exposure, 50 µl of the 5% RBCs were added to each well and the plates were gently agitated and then incubated at approximately 37°C for 3 hours. Agglutination appears as a diffuse red disc across the entire bottom of the well, whereas a lack of agglutination appears as a "button" at the bottom of the V-shaped well [9]. Titers were determined as the log 2 of the reciprocal of the highest dilution showing agglutination, which measures the activity of total hemagglutinating antibodies.

5.6.4 Assessment of Male Copulatory Behavior

Male copulatory behavior is a sensitive measure of endocrine disruption. Initiation of normal male sexual behavior is important for successful reproduction. When 90% of control males reached reproductive maturity (week 7 in both the F0 and F1 generations), copulatory behavior was assessed. To do so, reproductively naïve males and females were paired for three minutes on three consecutive days and observed continuously. Time to mount, number of mount attempts, and number of successful cloacal contacts were recorded. After behavioral assessment in the F0 generation, male and female quail were paired regularly (e.g., Monday, Wednesday, Friday) to produce the F1 generation. After behavioral assessment in the F1 generation, male and female quail were paired similarly to produce eggs. F1 eggs were incubated until ED 4, at which point eggs were opened to assess fertility.

5.6.5 Assessment of Fertility

One week prior to necropsy, eggs were collected from each egg-laying female from the F0 generation. Eggs were incubated in a Kuhl® 960 Model Automatic Turning Incubator with Digital Output at 99.5-100ºF and a relative humidity of 50-70% until ED14. Then, eggs were set to hatch in a Kuhl® 800 Model Double Cabinet Hatcher at 98.6-99.5ºF and a relative humidity of 70-75%. Hatched eggs were recorded as fertile. Three days after the last chick hatched, unhatched eggs were opened and fertility was evaluated. Eggs that did not have an embryo were classified as infertile. Eggs that did have an embryo were classified as fertile and sub-classified as either week 1, week 2, or ED18, according to their development. Eggs generated by un-mated females were excluded from fertility assessment.

One week prior to necropsy, eggs were collected from each egg-laying female from the F1 generation. Eggs were incubated in a Kuhl® 960 Model Automatic Turning Incubator with Digital Output at 99.5-100 (ºF) and a relative humidity of 50-70% until ED14, at which point eggs were opened and evaluated as fertile or infertile, based on the presence or absence of an embryo. Eggs generated by un-mated females were excluded from fertility assessment.

5.6.6 Assessment of Eggshell Strength and Thickness

Eggshell strength was measured to at least 0.001 kg of pressure using an Orka Technology® Egg Force Reader. Each egg was placed on its apex in the test stand so that the compression head made contact with the base of the egg. Eggshell thickness was then measured at a minimum of three points around the girth using calipers (Chicago Brand) that measured to at least 0.001 mm. Eggshell strength and thickness were measured for at least two eggs per female (when available), and the mean values were then calculated per bird.

5.6.7 Necropsy and Organ Mass

After 12 or 10 weeks of dosing the F0 and F1 generations, respectively, all surviving quail were bled via the jugular vein and euthanized using carbon dioxide (CO_2) and subsequent decapitation. Acute phase birds were similarly euthanized but neither blood nor tissues were collected. Necropsy for the F0 generation was scheduled over three days and necropsy order was randomized across dose groups. The F1 generation necropsy was similarly randomized, but scheduled across four days. When possible, blood and tissues were also taken prior to euthanizing moribund animals. Quail that died during the course of the study were submitted for gross necropsy if the bird was determined to have died recently. Similarly, tissues that were not grossly autolytic were submitted for histopathological evaluation.

A full, detailed gross necropsy, including a careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents was performed on all experimental animals following euthanasia. At necropsy, the brain, heart, kidneys, liver, ovaries, oviduct, bursa, cloaca, thyroids, spleen, testes, and epididymides were removed, trimmed, and weighed (except the kidneys and cloaca). Thyroids and testes were weighed as pairs. Any observed lesions were retained for processing.

The brain, heart, kidneys, liver, ovaries, oviduct, bursa, cloaca, thyroids, and spleen were stored in 10% buffered formalin for fixation. The testes and right epididymis from each animal were placed in Davidson's fixative overnight (no longer than 24 hours). After fixation, the tissues were rinsed with deionized water and stored in 70% ethanol.

5.6.8 Sperm Analysis

The left epididymis from each male was removed during necropsy and submitted for sperm analysis. In preparation, 10 ml of Gibco® Medium 199 (M199) was pipetted into each well of Corning® Costar® 6-well cell culture plates and warmed on a slide warmer to approximately 37ºC. Each epididymis was weighed, placed in a well containing M199, minced using small scissors, and incubated for 10 minutes at approximately 37ºC. Then, large pieces of tissue were removed, samples were swirled to mix, and 100 µl were transferred to labeled tubes containing 100 µl of IDENT® stain. Samples were incubated in IDENT for at least 10 minutes at approximately 37ºC prior to being loaded on a standard count chamber slide (Leja®). Slides were loaded into a Hamilton-Thorne IVOS Sperm Analysis System® and read using the IDENT® program.

5.6.9 Histopathology

As described in Appendix N, all tissues, with the exception of testis and epididymis, were fixed in formalin, trimmed into cassettes, processed, embedded in paraffin, sectioned via a microtome to a thickness of 4-5 um, and stained with hematoxylin and eosin using a routine automatic stainer. Testis and epididymis were fixed in modified Davidson's fixative for 24 hours, rinsed in deionized water, stored in ethanol, processed, paraffin-embedded, and sectioned identically to the other tissues, and stained with periodic acid-Schiff stain.

5.6.10 Clinical Chemistry and Hematology

Whole blood samples were collected from scheduled euthanasia/necropsy birds and placed in Sarstedt lithium heparin microtubes. When possible, blood samples were collected from moribund animals prior to euthanasia. Aliquots were collected for determination of hematocrit (HCT), total

solids, and hemoglobin concentration (HGB) with a refractometer and HemoCue®. The remaining sample was centrifuged on a Stat-Spin (hand-spin setting), plasma was collected, and electrolyte/clinical chemistry was performed on an Idexx VetTest® 8008 Chemistry Analyzer and the VetLyte® Electrolyte Analyzer.

5.7 Data Collection and Statistical Analyses

Data generated during the course of this study were recorded by hand and tabulated, summarized, and/or statistically analyzed using Microsoft® Excel, Minitab®, or SPSS® 21.0. Environmental data for the animal rooms was automatically recorded using MetaSys® Building Management System. Statistical significance was defined at the $p < 0.05$ level. Analyses were conducted for males and females separately. Parameters measured multiple times (i.e., body mass, cloaca gland, egg production) were measured using repeated measures ANOVA and those measured at the end of the study (i.e., organ weights) were analyzed using one-way analysis of variance (ANOVA) with dose group as the main effect. If the dose effect in the ANOVA was significant, Tukey and Dunnett post-hoc tests were used.

6 Results

6.1 Analytical Chemistry

The analytical chemistry results are summarized in Appendix D. All results were within the 70-130% recovery limits for the analysis. As such, all results are reported using the nominal concentrations. Stability analyses indicated that storage times and conditions were acceptable. Stability analyses were performed during a prior study with the same compound and diluent.

6.2 Avian Acute Oral Toxicity Test

Approximately 24 hours after dosing at the Limit Dose (2000 mg/kg), one female was ataxic, exhibited tremors, and showed signs of neurological toxicity (head tilt; opisthotonos). All other treated birds appeared normal. By approximately 48 hours after dosing, all five birds from the Limit Dose group appeared normal. No other clinical signs were seen through the remainder of the 14-day observation period. No mortality occurred in birds from the Limit Dose group.

Seven days post treatment, one male from the control group was found dead, of undetermined cause. In accordance with OECD guidelines, an additional five control birds were added to the test. The four original control birds were maintained for 14 days and had no adverse clinical signs or mortality. The five additional control birds were maintained for 14 days and had no adverse clinical signs or mortality.

6.3 One-Generation Study

6.3.1 Clinical Observations and Body Mass

Following 5 days of repeated oral exposure, parental generation (F0) birds from the 1000 mg/kg-day group began displaying neuromuscular signs including loss of balance and an inability to stand. Birds exhibited convulsions, circling on the floor of the cage, backward arching of the neck (opisthotonos), and alternated between prostrate inactivity and ataxic wing activity beginning 3-4 hours after dosing. Initially, neuromuscular activity ceased within a few hours. However, with

repeated daily dosing, periods of neuromuscular activity became quite prolonged, suggesting a dose-response. As such, there were periods when animals had limited ability to get to food or water; decreased body mass gain was detected in birds as early as one week into exposure (see Appendix E). Ultimately, all of the 1000 mg/kg-day birds met euthanasia criteria and were sacrificed prior to the end of their dosing period.

Individuals from the 500 mg/kg-day group began exhibiting similar neuromuscular signs at day 17 of exposure. Similar to birds from the higher dose group, periods of neuromuscular activity initially ceased within a few hours, but later became quite prolonged. Ultimately, all but one animal from the 500 mg/kg-day dose group met euthanasia criteria and were sacrificed prior to the end of their dosing period.

No adverse clinical signs or NTO-related mortality occurred in the 20 or 100 mg/kg-day groups. There were no differences in body mass between the control, 20, or 100 mg/kg-day groups for either sex from the F0 generation.

In the F1 generation, mild neuromuscular signs (e.g., star gazing) were observed in 10% of 100 mg/kg-day animals. Body mass of females from the 100 mg/kg-day group were increased compared to controls on days 0 and 2 (see Appendix E). Additionally, body mass of females from the 20 mg/kg-day group were increased compared to controls at weeks 4 and 5. These differences were not considered to be biologically relevant. Body mass of males from the 20 mg/kg-day group were increased compared to controls at day 2 and from weeks 4-10. These differences were not considered to be biologically relevant.

6.3.2 Sexual Development

In the F0 generation, there were no differences between dose groups in weekly measurements of cloaca gland size (see Appendix F). Additionally, there were no differences in the time it took males to reach reproductive maturity (40, 39, and 42 days for control, 20, and 100 mg/kg-day birds, respectively). Females from the 20 mg/kg-day group reached reproductive maturity most quickly (at day 42) compared to controls (at day 50) and 100 mg/kg-day birds (45 days). These differences were not considered to be biologically relevant. Egg production was increased sporadically in animals exposed to NTO (see Appendix G). Changes were neither consistent nor occurred in a dose dependent manner.

In the F1 generation, cloaca glands of birds exposed to 100 mg/kg-day were significantly smaller than controls at weeks 7-10 but not at week 6. At week 6 (see Appendix F), cloaca glands from control, 20, and 100 mg/kg-day birds measured 127, 132, and 116mm^2, respectively. At week 7, cloaca glands from control, 20, and 100 mg/kg-d birds measured 204, 203, and 175 mm^2, respectively. At week 10, cloaca glands from control, 20, and 100 mg/kg-day birds measured 353, 332, and 276 mm^2, respectively.

In the F1 generation, there were no differences between dose groups in the time it took males to reach reproductive maturity (41, 43, and 44 days for control, 20, and 100 mg/kg-day birds, respectively). Similarly, there were no differences in the time it took females to reach reproductive maturity (46, 46, and 49 days for control, 20, and 100 mg/kg-day birds, respectively). By week 8 (when most females were laying eggs regularly), average weekly egg production was 6 eggs/female. There were no differences between dose groups in weekly egg production (see Appendix G).

6.3.3 Immune Function

Plasma samples from F0 and F1 generation birds intended to assess immune function were compromised when the -30°C freezer they were being stored in was inadvertently shut off and the samples reached room temperature for an undetermined amount of time.

6.3.4 Male Copulatory Behavior

In the F0 generation, the only difference in copulatory behavior between animals was an increased number of mount attempts in the 100 mg/kg-day group compared to controls and the 20 mg/kg-day birds on day 2 (of 3 days) of testing (see Appendix H). No differences occurred in the time it took for males to mount females (mount lag), time to a successful copulation (success lag), or the number of successful copulations between groups. The time it took males to mount females (mount lag) decreased with each day of testing across dose groups. Other parameters were unchanged across days of testing.

In the F1 generation, no differences in mount lag, success lag, mount attempts, or successful copulations occurred between groups (see Appendix H). The time it took males to mount females (mount lag) and the time to the first successful copulation (success lag) decreased with each day of testing across dose groups.

6.3.5 Fertility

Fertility was unaffected in the F0 and F1 generation Japanese quail (see Appendix I). In the F0 generation, 89, 97, and 87% of eggs collected from control, 20, and 100 mg/kg-day birds, respectively, were fertile. In the F1 generation, 98, 93, and 91% of eggs collected from control, 20, and 100 mg/kg-day birds, respectively, were fertile.

6.3.6 Eggshell Strength and Thickness

Eggshell strength (Appendix J) and eggshell thickness (Appendix K) were unaffected in the F0 and F1 generation Japanese quail. In the F0 generation, eggs from control, 20, and 100 mg/kg-day birds were able to resist 1.11, 1.24, and 1.16 kg, respectively. On average, eggs from the F0 generation birds were 0.226 mm thick. In the F1 generation, eggs from control, 20, and 100 mg/kg-day birds were able to resist 1.26, 1.20, and 1.13 kg, respectively. On average, eggs from F1 generation birds were 0.215 mm thick.

6.3.7 Necropsy and Organ Mass

In the F0 generation animals that survived to scheduled necropsy, there were no differences in absolute organ mass, organ-to-brain mass, or organ-to-body mass (see Appendix L). In the F1 generation animals, there were no differences in absolute organ mass, organ-to-brain mass, or organ-to-body mass.

6.3.8 Sperm Analysis

The concentration of sperm was unaffected in the F0 and F1 generation Japanese quail (see Appendix M). In the F0 generation, mean sperm concentrations were 6.896, 5.675, and 5.907 million sperm/ml from control, 20, and 100 mg/kg-day birds, respectively. In the F1 generation, average sperm concentrations were 3.358, 3.394, and 3.560 million sperm/ml from control, 20, and 100 mg/kg-day birds, respectively. Of note, F0 birds were necropsied at 12 weeks of age; F1 birds were necropsied at 10 weeks of age.

6.3.9 Histopathology

Histopathology could not determine the cause of death in F0 generation birds from the 500 and 1000 mg/kg-day groups (see Appendix N). In one pathologic examination, the brains of 11 of 14 male birds exposed to 1000 mg/kg-day and 12 of 14 male birds exposed to 500 mg/kg-day exhibited vacuoles in the deep cerebellar nuclei and white matter of the same region. This finding was not present in the longer-lived male control birds and was present to a lesser extent in females.

Vacuolization of the cerebellum and/or the brainstem was observed following a histopathological re-examination by a second American College of Veterinary Pathology board certified military veterinary pathologist, and these changes were present in a dose dependent manner (see Appendix O). Vacuoles within the cerebellum were observed in all sections examined for the birds in the two highest dose groups. The cerebellum was not viable for examination for several due to processing related loss or it was not present in the plane of section. Three of the high-dose group birds (1000 mg/kg-day) had brainstem, but not cerebellum, available for examination. Within 4 of 12 males and 3 of 12 females in the mid-low dose group (100 mg/kg-day) vacuoles were noted in brainstem nuclei, but not the deep cerebellar nuclei.

Neuropil vacuoles (often presumed to be a histological processing artifact) were not noted in any animal in the low dose group (20 mg/kg-day) or the control group. Four birds, one male control and three from the lowest dose group (20 mg/kg-day) were sacrificed/died prior to the end of the study, and vacuoles were not observed in these birds. These birds were euthanized on days, 31, 32, 56, and 46 days of age, respectively. Additionally, one male bird in the mid-high (500 mg/kg-day) dose group survived until the end of the study and vacuoles were present in the cerebellum and brainstem, and these vacuoles were similar in distribution and severity to the birds that were sacrificed/died prematurely. The vacuoles within the grey matter neuropil are 25-40 microns in diameter, are empty or contain a small amount of eosinophilic material, and have regularly round smooth edges. The edges of the vacuoles are frequently located immediately adjacent to the neuronal cell body or capillaries. One female bird in the 20 mg/kg-day dose group had a focal area with cerebellar heterotopia or dysplasia, characterized by inversion of the normal cerebellar layers with normal appearing neurons in the incorrect location. This is likely an incidental finding that is unrelated to the study treatment or design.

It could not be determined if daily exposures to NTO at 500 and 1000 mg/kg-day affects testicular development in juvenile Japanese quail. One relatively age-matched control was available with which to compare the 500 and 1000 mg/kg-day exposure male F0 birds (e.g., those that exhibited neuromuscular signs and were euthanized early); testes from these birds were less mature than testes of the 31-day old control bird; however a single control bird cannot adequately represent the population. Similarly, the 500 mg/kg-day male, which survived the full 86 days, had moderately reduced testes compared to an 86-day old control, but a single control bird makes interpretation difficult. Testes of eleven F0 birds exposed to 100 mg/kg-day NTO, all of which survived to the end

of the study (85-87 days of age), were comparable in size and morphology to the age-matched control birds.

6.3.10 Clinical Chemistry and Hematology

In F0 generation animals that survived to scheduled necropsy, there were no differences in hematology (see Appendix Q). The only difference in clinical chemistry was decreased sodium (147.4 mmol/L) in 100 mg/kg-day males compared to controls (149.8 mmol/L) and 20 mg/kg-day (149.6 mmol/L) birds (see Appendix P).

In F0 animals that exhibited neuromuscular signs and were euthanized early, some clinical chemistry parameters were different compared to 12-week-old control animals, which may suggest an electrolyte imbalance. However, the sample size was small and age-matched controls were unavailable. In-house historical data on Japanese quail clinical chemistry shows high inter- and intra-strain variation.

In F1 generation animals, there were no differences in clinical chemistry (see Appendix P). The only difference in hematology was decreased total solids in 100 mg/kg-day females (5.62 g/dL) compared to controls (7.07 g/dL) and 20 mg/kg-day (6.00 g/dL) birds (see Appendix Q).

6.4 Determination of Benchmark Dose

Mortality was identified as the critical endpoint in this study. Benchmark Dose Software (BMDS 2.6.0.1) was used to fit mathematical models to mortality data and calculate a lower-bound confidence limit on a dose corresponding to a 10 percent response rate ($BMDL_{10}$) [10]. Data for male and female F0 generation quail were run separately and concurrently. Ultimately, the Logistic, Log-Logistic, Log-Probit, Probit, and Weibull models were selected based on goodness-of-fit and statistical parameters ($p>0.1$, lowest AIC values and residuals). A mean BMD of 348 mg/kg-day was derived for male and female F0 generation quail based on the results of these five models. This corresponded to a $BMDL_{10}$ of 151 mg/kg-day for male and female F0 generation quail.

7 Discussion

In prior oral toxicity studies using rodents, NTO-induced mortality was not observed [2]. In a 90-day oral toxicity study with rats, the most pronounced effects of NTO exposure were decreased testicular and epididymal mass and decreased sperm count. In the present study, mortality through an observed neuromuscular mode of action that was not explained by histopathology was the predominant effect and could have masked the concentration at which similar adverse testicular effects may have occurred. The extended one-generation test in Japanese quail yielded the following results: daily oral exposure to NTO at 500 and 1000 mg/kg-day induced neuromuscular effects and compound-related pre-term mortality. However, this is not the first time convulsions have been observed in response to munitions or in birds. Frank convulsions, caused by inhibition of GABAA in the brain, have been observed following experimental RDX exposure in a range of species, including rodents, non-human primates, lizards, and birds [11-15].

Williams et al., observed myoclonic twitches, 3-4 clonic-tonic seizures, and death 2-3 hours following RDX administration in rats [16]. Blood and brain concentrations of RDX during seizures indicated a direct correlation; i.e., the higher the concentration of RDX in the brain, the quicker the onset to seizures. Given that RDX readily crosses the blood-brain barrier and has a direct correlation with seizure onset [16] and that NTO exhibits testicular toxicity [2-5, 7], NTO may be

crossing the blood-testis and/or blood-epididymis barriers. The blood-testis and blood-epididymis barriers consist of the physical barrier formed by tight junctions between Sertoli cells in addition to physiological and immunological components [17]. In contrast, the blood-brain barrier is located at endothelial tight junctions lining blood vessels, [18] which make the blood-brain barrier relatively less permeable than the blood-testis/epididymis barriers.

With RDX, neurological effects were observed among a myriad of species and attributed to binding at the $GABA_A$ receptors [11-16]. Neurological signs were not observed following experimental exposure of rodents and non-human primates to NTO [2-5, 7, 19]. However, the putative neurological effects observed with the high doses would suggest that NTO penetrates the blood-brain barrier, assuming that there is no indirect or physiological effect (i.e., electrolyte imbalance). Since convulsions were observed in the current study, another plausible explanation is that Japanese quail (and perhaps other birds) have a unique receptor, metabolite, and/or pathway, albeit one with a low affinity for NTO; this would mean there is less of a concern for neurological effects across species. If birds have a unique feature that makes them susceptible to NTO-induced convulsions, this could be an issue for a water-soluble contaminant like NTO, which can easily mobilize into water. However, the very high effective doses found to cause convulsions in this study would be unlikely to be found in water. In conclusion, knowing that a receptor, metabolite, or pathway is specific to birds would be beneficial from the perspective of human health and risk assessment.

The vacuoles observed in this study are most likely present within astrocyte processes and cytoplasm [20]. The exact mechanism for which the vacuoles developed is unknown, but the development of the vacuoles is most likely secondary to exposure of the test article. As vacuoles were not seen in the control groups, common interpretation that neuropil vacuoles are simply the result of histological processing methods can be ruled out. Similar to the effects of ammonia on astrocytes from hyperammonemia [21-24], the test article may have caused increased activation or metabolism process by astrocytes resulting in the development of the vacuoles.

Other munitions, specifically nitroaromatic compounds (including RDX) cause neurologic deficits. Oral exposure to RDX causes grand mal seizures, which has been shown to be mediated through inhibition of the GABAA receptor [16], but neurohistopathologic lesions were not apparent after 13 weeks of oral exposure in rats [25] or after 14 day exposure in northern bobwhite quail [15]. Therefore a direct histopathological comparison cannot be made between these toxicants. Additional investigation is warranted to elucidate the mechanism by which NTO induces the neurologic deficits and vacuoles within the cerebellum and brainstem observed in these birds and whether these are causative or associative in the observation of neuromuscular spasms. It should be pointed out that convulsions have not been verified by EEG, though they were confirmed in a preliminary observation by a seizure expert [26]. Neither brain lesions nor convulsions have been seen in past work with NTO in rodents.

Differences in kidney function or metabolism between birds and other species exposed to NTO may help explain the differences in results. Serial blood draws from NTO-exposed rhesus macaques (*Macaca mulatta*) indicated that peak blood absorption occurs within 5 hours [19]. Similarly, NTO was 100-fold more concentrated in the urine, indicating that it is excreted quickly via the kidneys and does not undergo significant hepatic metabolism/recirculation. In rats exposed to NTO via oral gavage, blood NTO levels peak within 2 hours of dosing and NTO is excreted rapidly un-metabolized in urine and is no longer detected 8 hours after dosing. Because of its high water solubility and the speed with which it is excreted in rhesus macaques, it is unlikely that NTO is metabolized by the liver. However, of the two proposed NTO metabolites mediated by cytochrome P450 [27], one was detected at very low concentrations in urine of macaques [19]. It is uncertain if

this same metabolism and excretion profile also applies to birds, which may account for the lack of clinical signs seen in mammalian species vs those observed in this study.

8 Conclusions

Repeated oral exposures to 500 and 1000 mg/kg-day-NTO induced neuromuscular signs and compound-related pre-term mortality in male and female Japanese quail. Birds exhibited convulsions, circling on the floor of the cage, backward arching of the neck (opisthotonos), and alternated between prostrate inactivity and ataxic wing activity. In conjunction with neuromuscular signs, decreased body mass gain occurred in birds as early as one week into exposure. Ultimately, all of the 1000 mg/kg-day birds and all but one of the 500 mg/kg-day birds met euthanasia criteria and were sacrificed. Histopathology could not determine the cause of death in F0 generation birds from the 500 and 1000 mg/kg-day groups. However, vacuolization of the cerebellum and/or the brainstem was observed and these changes were present in a dose-dependent manner. It is uncertain at this stage if these vacuoles are causative or merely associated with convulsions. Mild neuromuscular signs occurred in F1 generation birds from the 100 mg/kg-day group but not from birds in the 20 mg/kg-day group or controls in either generation. No other sublethal adverse effects were observed.

Mortality was identified as the critical endpoint in this study. A mean BMD of 348 mg/kg-day was calculated for male and female F0 generation quail based on the results of the 5 BMDL models. This corresponded to a $BMDL_{10}$ of 151 mg/kg-day for male and female F0 generation quail. Data from an extended one-generation study in rats exposed to NTO via drinking water were used to derive $BMDL_{10}$ values ranging from 2335-2775 mg/L (140-160 mg/kg-day) for reproductive effects in F0 generation males (e.g., epididymal mass and sperm count) and 1048-2794 mg/L (120-310 mg/kg-day) for reproductive/developmental effects in F1 males (e.g., nipple retention, pre-pubertal separation, testes mass, epididymal mass, seminal vesicle and coagulating gland mass) [7]. Neither brain lesions nor convulsions have observed seen in previous toxicology studies in rodents exposed to NTO.

9 Point of Contact

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Appendix A

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Appendix B

Quality Assurance Statement

For: Toxicology Study No. S.0027395.3, Protocol No. 80-14-07-02, One-generation reproductive toxicity test in Japanese quail *(Coturnix japonica*) using to 3-Nitro-1,2,4-Triazol-5-One (NTO), February 2015-August 2015, the following critical phases were audited by the Quality Systems and Regulatory Compliance Office's Quality Assurance Unit:

PRE IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Study Protocol Good Laboratory Practice Standards and Animal Care Review	06/27/2014	06/27/2014

IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Acute Oral Toxicity Test–Incubator temperature/humidity settings & Protocol modification compliance	01/02/2015	01/09/2015
Acute Oral Toxicity Test – Egg Veterinary Care, Hatcher sanitation, temperature and alarm procedures	01/05/2015	01/14/2015
Acute Oral Toxicity Test - Test System Facilities, Identification, Husbandry & Food and Water Supply	02/13/2015	02/18/2015
Acute Oral Toxicity Test-Maintenance and Calibration of Equipment and Good Documentation Practices	02/13/2015	02/18/2015
Study Personnel Qualifications and Training Records - Pre-study training requirements verification	02/18/2015	02/25/2015
Acute Oral Toxicity Test - Sub-study Endpoint Criteria Compliance	03/4/2015	03/10/2015
F0 Generation - Maintenance and Calibration of Equipment and Good Documentation Practices	03/17/2015	03/26/2015
F0 Generation - Test System - Facilities, Identification, Husbandry, Feed and Water & Enrichment	03/17/2015	03/24/2015
F0 Generation - Test Article Storage, Control, Mixing, Labeling and Administration	03/17/2015	03/26/2015
F0 Generation - Assessment of Sexual Development/Fertility and Behavioral Assessment/Mating	04/16/2015	04/23/2015
F0 - Generation Pre and Post Procedural Provisions and Husbandry Considerations	04/16/2015	04/23/2015

Analytical Chemistry Support - Dosing Solution Concentration Verification Procedures and Data Review	04/29/2015	05/07/2015
F0 Generation Euthanasia and Biosample Collection Procedures	05/21/2015	05/28/2015
F0 Generation Necropsy, Organ Sample Collection, Sperm Analysis and Sub-Study Endpoint Procedures	05/21/2015	05/28/2015
F1 Generation - Test System - Feed & Water Supply, Identification, Husbandry Facilities & Procedures	07/15/2015	07/22/2015

For: Toxicology Study No. S.0027395.3, Protocol No. 80-14-07-02, One-generation reproductive toxicity test in Japanese quail *(Coturnix japonica*) using to 3-Nitro-1,2,4-Triazol-5-One (NTO), February 2015-August 2015, the following critical phases were audited by the Quality Systems and Regulatory Compliance Office's Quality Assurance Unit:

IN-LIFE PHASE OF THE STUDY (continued)

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
F1 Generation - Administration of Test Substance - Oral Gavage Technique Procedures	07/15/2015	07/22/2015
F1 Generation - Test Article control, mixing and labeling and compliance with facility SOPs	07/15/2015	07/22/2015
Behavioral Assessment/Mating and Test System Observations	07/30/2015	07/31/2015
F1 Generation - Maintenance and Calibration of Equipment and Good Documentation Practices	08/12/2015	08/24/2015
F1 Generation - Pre and Post Procedural Provisions and Husbandry Considerations	08/12/2015	08/24/2015
F1 Generation Euthanasia and Biosample Collection Procedures	08/18/2015	08/24/2015
F1 Generation Necropsy, Organ Sample Collection, Sperm Analysis and Sub-Study Endpoint Procedures	08/18/2015	08/24/2015

POST IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Pathology Contributing Scientist Inspection-Interim Pathology Report GLP Standard Regulation Review	08/17/2016	08/24/2016
Pathology Contributing Scientist Inspection - Interim Report	08/17/2016	08/24/2016

Summary Data and Summary Table Review		
Pathology Contributing Scientist Inspection - Final Report Summary Data and Summary Table Review	09/14/2016	09/14/2016
Pathology Contributing Scientist Inspection - Final Study Raw Data GLP Standard Regulation Review	09/14/2016	09/14/2016
Pathology Contributing Scientist Inspection - Final Report Summary Data and Summary Table Review	09/22/2016	09/23/2016
Pathology Contributing Scientist Inspection- Final Pathology Report GLP Standard Regulation Review	09/22/2016	09/23/2016
Pathology Addendum Report Inspection -Final Report Summary Data and Summary Table Review	03/18/2017	04/11/2017
Pathology Addendum Report Inspection - Final Study Data GLP Standard Regulations Review	03/18/2017	04/11/2017
Final Study Report Good Laboratory Practice Standards Review	05/08/2017	05/10/2017
Final Study Raw Data GLP Standards Review	05/08/2017	05/10/2017

For: Toxicology Study No. S.0027395.3, Protocol No. 80-14-07-02, One-generation reproductive toxicity test in Japanese quail *(Coturnix japonica)* using to 3-Nitro-1,2,4-Triazol-5-One (NTO), February 2015-August 2015, the following critical phases were audited by the Quality Systems and Regulatory Compliance Office's Quality Assurance Unit: ***(Notes)***

Note 1 *All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.*

Note 2 *In addition to the study specific critical phase inspections listed here, general facility and process based inspection not specifically related to this study are done monthly or annually in accordance with QA Standard Operating Procedure.*

Note 3 *This report has been audited by the Quality Assurance Unit (QSARC), and is considered to be an accurate account of the data generated and of the procedures followed*

Michael P. Kefauver
Quality Assurance Specialist, QSARC-QAU

05 JULY 2017
Date

Appendix C

Archives and Study Personnel

C-1 Archives

All raw data, documentation, records, protocol, and a copy of the final report generated as a result of this study will be archived in room 1026, building E-2100, APHC, for a minimum of 10 years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

Records on animal receipt, diet, and facility environmental parameters will be archived by the Veterinary Medical Office for a minimum of 10 years following submission of the final report to the Sponsor.

Some ancillary records pertaining to this study, such as instrument maintenance logs, animal room observation logs, etc., will not be archived until those logbooks have been completed. Once complete they will be archived in room 1026, building E-2100, APHC.

Wet tissues, histology slides, and paraffin blocks are stored in building E-5158.

C-2 Personnel

Management: Dr. Mark S. Johnson, Ph.D., Director, Toxicology; Mr. Arthur J. O'Neill, Chief, Toxicity Evaluation Division (TEV); Dr. Michael J. Quinn, Ph.D., Chief, Health Effects Division (HEF).

Study Director: Allison M. Jackovitz, Biologist, TEV.

Quality Assurance: Michael P. Kefauver, Quality Assurance Specialist, Quality System and Regulatory Compliance.

Veterinary Support and Animal Care: Kenneth E. Despain, DVM, LTC, VC; Mary E. Sprangel, DVM, MAJ, VC; Robert Sunderland, Animal Health Technician; Rebecca Kilby, Animal Health Technician..

Pathology Lab Coordinator: Alicia A. Shiflett, Histotechnician, TOX-PATH.

In-Life Support: Allison M. Jackovitz, Biologist, TEV; Theresa L. Hanna, Biological Science Technician, TEV; Stephen W. Rice, Biological Science Technician, ORISE; Mark R. Way, Biologist, TEV; Adam T. Deck, Biologist, Environmental Health Risk Assessment Division; Michael J. Quinn, Biologist, HEF.

Necropsy: Mark R. Way, Biologist, TEV; Allison M. Jackovitz, Biologist, TEV; Michael J. Quinn, Biologist, HEF; Erica E. Carroll, Pathologist, TOX-PATH; Stephen W. Rice, Biological Science Technician, ORISE; Theresa L. Hanna, Biological Science Technician, TEV; Ryan M. Johnson, Biological Science Technician, ORISE; Emily May Lent, Toxicologist, TEV.

Clinical Chemistry: Matthew A. Bazar, Biologist, TEV.

Archivist: Martha L. Thompson, Data Acquisition Specialist, TEV.

Appendix D

Analytical Observations

NTO Sample Type	Date	Nominal Concentration (mg/ml)	Analytical Concentration (mg/ml)	% of Nominal
Stability Test: 3 weeks post preparation	4/9/2012	6	6.0	100%
Stability Test: 4 weeks post preparation	4/16/2012	6	6.3	105%
Stability Test: 5 weeks post preparation	4/23/2012	6	6.3	105%
Stability Test: 6 weeks post preparation	4/30/2012	6	6.3	105%
Stability Test: 7 weeks post preparation	5/7/2012	6	6.4	107%
Stability Test: 8 weeks post preparation	5/14/2012	6	6.3	105%
Concentration Verification	3/4/2015	2	1.86	93%
Concentration Verification	3/4/2015	10	8.59	86%
Concentration Verification	3/4/2015	50	46.1	92%
Concentration Verification	3/4/2015	100	88.0	88%
Concentration Verification	3/20/2015	2	1.80	90%
Concentration Verification	3/20/2015	10	8.88	89%
Concentration Verification	3/20/2015	50	45.6	91%
Concentration Verification	3/20/2015	100	87.9	88%
Concentration Verification	4/9/2015	2	1.89	95%
Concentration Verification	4/9/2015	10	10.4	104%
Concentration Verification	4/9/2015	50	42.5	85%
Concentration Verification	4/23/2015	2	1.77	89%
Concentration Verification	4/23/2015	10	8.88	89%
Concentration Verification	5/5/2015	2	1.93	97%
Concentration Verification	5/5/2015	10	8.37	84%
Concentration Verification	6/23/2015	2	1.93	97%

Concentration Verification	6/23/2015	10	8.88	89%
Concentration Verification	7/10/2015	2	2.01	101%
Concentration Verification	7/10/2015	10	9.44	94%
Concentration Verification	7/10/2015	10	9.83	98%
Concentration Verification	8/20/2015	2	1.90	95%
Concentration Verification	8/20/2015	2	1.90	95%
Concentration Verification	8/20/2015	2	2.00	100%
Concentration Verification	8/20/2015	10	9.5	95%
Concentration Verification	8/20/2015	10	8.5	85%
Concentration Verification	8/20/2015	10	9.4	94%

Appendix E

Individual and Summary Body Mass Data

Table E-1
12 Week Individual Body Mass (grams)
F0 Generation Female Quail

Dose Group	Animal ID	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
	Y1	65.7	103	131.8	148.5	175.3	192.8	174.3	161.6	179.7	181.7	187.6
0 mg/kg	Y4	67.2	106.3	138.6	160	191.8	190.2	189.8	197.1	199.7	195.7	192.4
	Y11	71.1	107.5	133.9	145.5	160.4	188.3	189.5	194.5	197.5	206.2	202.9
	Y22	68.6	102.8	132.7	156	173	193.4	183.7	170.4	190.5	179.8	185.6
	Y23	71.3	106	134.6	153.1	192.2	181.9	182.7	200.5	194.7	217	211.3
	Y24	70.6	109.4	135.2	131	158.2	155.9	154.5	145.2	170.8	170.9	174.5
	Y29	56.8	91.9	121.3	137.6	154.4	190.3	168	165.6	166.4	179.7	173.3
	Y30	62.8	95.8	124.8	143.4	180	190.1	187.6	192.3	185	178.9	170.6
	Y34	74.4	116.9	142.6	156	193.4	185	191.7	186.4	195.7	200.1	197.9
	Y46	71.4	112.7	144.7	162.6	179	185.1	182.1	194.7	198.3	202.4	207.2
	Y52	73.4	116.5	143.8	159.1	202.5	187.3	176.6	161.9	173.7	165.7	174.7
	Y59	65.2	107.5	131.1	156	198.9	197.6	194.2	199.5	208.1	MK	-
	Mean	**68.2**	**106.4**	**134.6**	**150.7**	**179.9**	**186.5**	**181.2**	**180.8**	**188.3**	**188.9**	**188.9**
	SD	**5.00**	**7.45**	**7.16**	**9.69**	**16.29**	**10.52**	**11.39**	**18.78**	**13.11**	**16.20**	**14.58**
	P1	67.8	106.3	135	152.4	183.8	189.1	196.8	194.4	204.1	206.3	204.8
20 mg/kg	P4	66.5	102.5	128.4	148.4	124.5	MK	-	-	-	-	-
	P9	62.9	97.9	127.9	151.3	185.3	177.3	186.4	183.1	181.8	188.9	184.4
	P10	71.8	109.1	139.3	167	180.4	187.7	203.7	206.5	204.3	207.3	202.7
	P15	63	99.1	128.5	147.9	190.9	189.3	125.3	MK	-	-	-
	P16	71.4	109	137.9	162.3	202.5	199.4	192.3	197.1	200.3	200.4	208.9
	P17	70.8	110.7	142.2	155.5	189.9	194.6	201.3	192.6	202.1	196.8	204.5
	P22	72.2	111.1	135.3	159.1	203.7	201.4	184.6	194.9	200.6	205	207.2
	P24	69.8	110.2	136.4	158	188.3	177.7	184.7	190.9	190.8	192.1	191.3
	P31	59.3	96.5	124	136	163.8	194.5	194.3	184.2	190.4	188.1	165.6
	P39	60.7	95.3	124.4	139	159.9	170.7	167.9	176.8	175.6	166.3	178

	P42	70.3	104.5	133	155.9	157.5	184.5	193.4	185	191.2	190.7	204.8
	Mean	**67.2**	**104.4**	**132.7**	**152.7**	**177.5**	**187.8**	**184.6**	**190.6**	**194.1**	**194.2**	**195.2**
	SD	**4.62**	**5.91**	**5.96**	**9.01**	**22.52**	**9.64**	**21.96**	**8.51**	**9.86**	**12.20**	**14.77**
	G7	53.4	88.1	115.5	143.2	155.4	163	167.7	164.9	170.7	172.9	173
100 mg/kg	G11	64.5	101	131.7	150.6	183.3	194.7	201.1	197.2	202.7	211.4	207.3
	G14	68.1	102.8	130.8	144.8	157.5	181.8	179.2	170.9	178.8	187.6	193.2
	G15	57.2	89.9	122.1	141	173.4	191.8	192.3	195.4	192.3	205.4	197.8
	G18	64.4	101.5	135.8	150.3	171.8	208.2	193.9	196	200.1	198.2	202.6
	G26	74.1	110.5	137.6	161.7	197.3	213.7	207.5	213.7	216.4	218.4	208.8
	G30	68.7	105.4	129.2	140.8	179.5	189.1	188	178.2	185.4	188.2	185.1
	G31	78.1	119.2	152.3	176	209.2	223	221	229.7	234.1	234.1	238.9
	G35	64.7	104.2	131.9	146.3	168.2	192.1	186.7	180	182.1	183.6	186.5
	G38	68.1	104.6	136	152.6	176.3	169.1	173.1	176.1	185.6	183.8	187.9
	G40	67.5	101.5	129	155.2	194.6	176.5	186.7	186	178.1	192.2	193.5
	G42	69.9	105.4	137.7	155.5	183.4	196.2	203.7	209.8	216	218.9	219.7
	Mean	**66.6**	**102.8**	**132.5**	**151.5**	**179.2**	**191.6**	**191.7**	**191.5**	**195.2**	**199.6**	**199.5**
	SD	**6.64**	**8.18**	**9.01**	**10.01**	**15.82**	**17.67**	**15.04**	**19.22**	**19.08**	**18.19**	**17.60**
	B1	68.3	101.8	128.2	134.1	140.9	MK	-	-	-	-	-
500 mg/kg	B11	57.1	90.4	119	MK	-	-	-	-	-	-	-
	B15	60	94.7	120.5	128.4	MK	-	-	-	-	-	-
	B16	60.9	96.5	122	106.8	MK	-	-	-	-	-	-
	B17	67.8	104.6	133.9	MK	-	-	-	-	-	-	-
	B19	66	93.3	123.1	129.1	136.2	135.6	MK	-	-	-	-
	B21	61.8	93.2	117	125.9	110.8	MK	-	-	-	-	-
	B23	69	102	130.3	MK	-	-	-	-	-	-	-
	B25	56.3	88.9	113.2	89.2	MK	-	-	-	-	-	-
	B26	68.8	109.4	134.6	MK	-	-	-	-	-	-	-
	B27	61.8	92.1	119.3	MK	-	-	-	-	-	-	-
	B28	66.3	102.5	130.9	MK	-	-	-	-	-	-	-
	B30	64.6	92.3	115.6	MK	-	-	-	-	-	-	-
	B31	69.1	105.2	135.6	143.1	143.9	MK	-	-	-	-	-

	B33	76.6	114	142.2	MK	-	-	-	-	-	-	-
	B35	59.5	90.2	116.3	MK	-	-	-	-	-	-	-
	B37	75	115	141.4	159.8	184.8	175.3	MK	-	-	-	-
	B38	63.7	95.2	119.1	114.7	117.1	MK	-	-	-	-	-
	B40	56.4	88	118	109	MK	-	-	-	-	-	-
	B41	69.6	105.9	132.1	MK	-	-	-	-	-	-	-
	Mean	**64.9**	**98.8**	**125.6**	**124.0**	**139.0**	**155.5**	**-**	**-**	**-**	**-**	**-**
	SD	**5.76**	**8.29**	**8.94**	**20.04**	**26.11**	**28.07**	**-**	**-**	**-**	**-**	**-**
	O2	66.7	99.7	MK	-	-	-	-	-	-	-	-
1000 mg/kg	O6	65	96.6	MK	-	-	-	-	-	-	-	-
	O8	62	97	110.3	112.2	MK	-	-	-	-	-	-
	O11	58.3	89	107.6	107.9	MK	-	-	-	-	-	-
	O13	70.5	92.9	MK	-	-	-	-	-	-	-	-
	O16	67.7	96.6	MK	-	-	-	-	-	-	-	-
	O17	62.3	93.4	MK	-	-	-	-	-	-	-	-
	O22	77.5	119.5	134.8	MK	-	-	-	-	-	-	-
	O24	65.8	92.7	MK	-	-	-	-	-	-	-	-
	O26	60.6	93.4	MK	-	-	-	-	-	-	-	-
	O28	63.6	95.6	MK	-	-	-	-	-	-	-	-
	O29	73.5	102.1	MK	-	-	-	-	-	-	-	-
	O31	66.9	95.1	100.8	MK	-	-	-	-	-	-	-
	O36	60.8	96.2	117.5	MK	-	-	-	-	-	-	-
	O38	68.4	104.5	134.9	MK	-	-	-	-	-	-	-
	O43	72	103.2	105.9	118.3	MK	-	-	-	-	-	-
	O46	73.5	106.8	MK	-	-	-	-	-	-	-	-
	O47	74.9	115.1	MK	-	-	-	-	-	-	-	-
	O52	62.5	94.4	109.4	MK	-	-	-	-	-	-	-
	O53	65.1	100.9	121.5	MK	-	-	-	-	-	-	-
	O54	69.9	100.3	MK	-	-	-	-	-	-	-	-
	O56	76.2	111.4	121.2	126	MK	-	-	-	-	-	-

O58	62.5	96.1	100.4	MK	-	-	-	-	-	-	-
O60	56.9	86.3	117.8	115.4	MK	-	-	-	-	-	-
O61	75	112.7	141.5	MK	-	-	-	-	-	-	-
O63	62.4	90.1	105.7	MK	-	-	-	-	-	-	-
Mean	**66.9**	**99.3**	**116.4**	**116.0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
SD	**5.80**	**8.25**	**13.16**	**6.81**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

MK = moribund kill

Table E-2
12 Week Individual Body Mass (grams)
F0 Generation Male Quail

Dose Group	Animal ID	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
	Y2	61.6	92.8	118.5	130.8	137	139.5	139.4	141.8	143.6	144.6	146.1
0 mg/kg	Y7	62.6	99.8	127.6	138.5	138	147.6	150	153.8	153.5	154.5	155.8
	Y8	68.6	107.5	136.4	153.9	159.3	165	163.6	167.3	164.2	166.3	165.5
	Y10	69	107.2	131.6	143.7	150.6	156.5	147.9	154.8	149.8	153	156.6
	Y19	65.6	102.8	129	151	162.6	168	162.2	166.2	164.5	161.6	166.5
	Y21	62.3	99.5	129.3	141.9	146.6	157.1	MK	-	-	-	-
	Y26	77.2	119.7	145.2	152.8	155.5	161.3	158.1	156.2	161.2	163.2	164.9
	Y35	61.3	94.9	121.7	141.7	149.3	146.7	150.9	152.6	154.1	163.9	164.5
	Y38	51.4	85	109.7	130.9	134.2	141.9	142.2	144.7	148.8	151.4	151.9
	Y39	67.5	101.4	131.9	143.3	147.5	143.4	157.1	158.5	159	160.9	166.6
	Y42	68.7	106.4	135.8	152.3	160.7	162.9	166.6	172.9	163.5	166.7	169.5
	Y51	63.8	101.3	130.6	142.2	143.6	148.3	147	152.7	152.4	153.1	157.2
	Mean	**65.0**	**101.5**	**128.9**	**143.6**	**148.7**	**153.2**	**153.2**	**156.5**	**155.9**	**158.1**	**160.5**
	SD	**6.20**	**8.65**	**9.14**	**7.87**	**9.50**	**9.78**	**8.95**	**9.38**	**7.06**	**7.16**	**7.38**
	P3	74.6	109.2	137.1	151.5	156.9	168.2	169.1	170.4	176.7	177.7	178.5
20 mg/kg	P5	62.8	94.7	128.6	144.4	152.4	122.5	157.2	161.8	166.4	172.1	168.4
	P12	61	89.5	123.1	146.1	160.1	159.6	163.5	162.1	152.9	156.6	159.7
	P14	63	97.6	122.5	135.4	141.6	147.1	145.3	148.2	148	147.5	150.2
	P18	72.4	109	135.8	149.6	153.5	163	166.2	171	175	175.8	176.1
	P19	66.1	103	128.5	135.8	144.4	151	152.2	153.9	154.6	158.1	159.4
	P20	70.4	111.2	141.1	156.1	166	175.9	174.6	181.2	178.7	183	182.5
	P21	60.1	98.4	127.2	142	142.8	142.6	143	150.7	151.8	154.1	158.7
	P26	70.5	110.1	120.2	149.9	156.8	160.3	165.1	166.1	170.7	175.1	170.3
	P34	73	113.6	139.7	158	163.9	171.9	163.2	172.7	173	180	180.2
	P38	71	108.2	133.8	145.6	145.8	155.5	156.3	161.2	161.6	167.6	165.8

	P40	73.8	113.5	138	153.8	157.4	162.9	163.3	167.9	169.9	168.4	168.9
	Mean	**68.2**	**104.8**	**131.3**	**147.4**	**153.5**	**156.7**	**159.9**	**163.9**	**164.9**	**168.0**	**168.2**
	SD	**5.30**	**7.99**	**7.21**	**7.25**	**8.23**	**14.54**	**9.44**	**9.66**	**10.77**	**11.41**	**9.96**
	G2	70.3	107	133.6	142.2	144.9	148.9	157.6	158.4	165	168.3	168.4
100 mg/kg	G8	60.5	98.6	126.9	140.6	151.5	159.3	160.4	165.9	168.1	171.8	171.6
	G9	55.7	91.8	116.7	134.2	133.3	137.2	134.7	135.4	140.2	143.1	144
	G17	61.1	99.1	130.6	147.8	156.3	163.9	164.5	168.7	169.2	172.4	171.9
	G19	71.9	114.3	146.4	164.5	158.9	163.8	160.5	164.9	171.5	170.9	176.7
	G20	64.1	98	127.4	141	145.8	149.3	151.6	158.1	160.8	166.3	166.2
	G23	66.6	102.3	135.1	153.7	161.7	164.6	162.2	142.5	MK	-	-
	G24	70.9	103.7	125.2	136.8	138.4	141.2	136	139.5	137.9	142	140.6
	G27	58.8	95.4	122.8	139.4	141.1	149.4	146.6	151.8	157.4	155.5	153.1
	G28	71.7	111.4	140.5	159.9	161.3	162.2	161.3	168.5	172.3	175.7	179.5
	G32	62.6	96.6	123	142.8	142.4	139.7	137.2	145.8	150.6	153.4	158.3
	G39	68.8	105.8	135.4	146.2	151.2	153.2	155.2	159.7	163.9	167.3	169.4
	Mean	**65.3**	**102.0**	**130.3**	**145.8**	**148.9**	**152.7**	**152.3**	**154.9**	**159.7**	**162.4**	**163.6**
	SD	**5.55**	**6.71**	**8.33**	**9.26**	**9.37**	**10.01**	**11.01**	**11.70**	**12.04**	**11.95**	**12.93**
	B2	58.6	90.5	115.7	103.7	125.6	MK	-	-	-	-	-
500 mg/kg	B3	53.1	82.7	111.5	109.5	136.6	MK	-	-	-	-	-
	B4	63.5	94.2	119.2	128.7	137.8	MK	-	-	-	-	-
	B5	60.6	93.3	115.2	126.8	MK	-	-	-	-	-	-
	B6	61.8	93.4	123.2	139.3	144.1	-	-	-	-	-	-
	B7	67	101.7	126.3	130.5	MK	-	-	-	-	-	-
	B8	66	101.9	127.8	143.3	128.1	-	-	-	-	-	-
	B9	57.9	91.5	118.9	131.4	MK	-	-	-	-	-	-
	B10	62.4	94	121.7	MK	-	-	-	-	-	-	-
	B12	63.2	96.7	128.8	137.4	MK	-	-	-	-	-	-
	B13	57.6	87.6	114.7	128.4	132.6	133	136.5	133	145.3	151.7	MK
	B14	76.1	108.8	139.4	MK	-	-	-	-	-	-	-
	B18	44.1	74.1	106.1	125.8	136.3	143.7	140.8	125.3	143.8	148.2	151.1
	B20	48.5	80	110.3	124.2	130.4	MK	-	-	-	-	-

	B22	72.3	113.2	136.7	149.4	MK	-	-	-	-	-	-
	B24	68.1	105.5	126.3	128	137.1	125.3	MK	-	-	-	-
	B29	67.7	100.2	129.5	123.4	140.4	137	MK	-	-	-	-
	B32	73.4	111.4	141.3	148.5	MK	-	-	-	-	-	-
	B34	62.8	95.2	117.3	131.4	132.8	MK	-	-	-	-	-
	B36	70.4	108.7	135.8	130.7	111.9	MK	-	-	-	-	-
	B39	62.4	99	124.9	136.8	132.9	MK	-	-	-	-	-
	B42	56.9	95.9	123.7	141	MK	-	-	-	-	-	-
	Mean	**62.5**	**96.3**	**123.4**	**130.9**	**132.8**	**134.8**	**138.7**	**129.2**	**144.6**	**150.0**	**151.1**
	SD	**7.79**	**9.99**	**9.52**	**11.28**	**8.02**	**7.69**	**3.04**	**5.44**	**1.06**	**2.47**	**-**
	O3	60.4	91.7	107.6	111.2	MK	-	-	-	-	-	-
1000 mg/kg	O4	60	86.6	MK	-	-	-	-	-	-	-	-
	O5	56.3	84.2	97.4	107.1	MK	-	-	-	-	-	-
	O10	64	93.6	99.7	MK	-	-	-	-	-	-	-
	O12	67.6	100	121.1	127.3	MK	-	-	-	-	-	-
	O14	66	97.9	117.6	MK	-	-	-	-	-	-	-
	O18	61	90.1	105.8	113.2	MK	-	-	-	-	-	-
	O19	66.2	99.5	121.9	125.4	MK	-	-	-	-	-	-
	O21	63.6	96.8	101.9	124.1	MK	-	-	-	-	-	-
	O23	66.4	103.3	126.2	MK	-	-	-	-	-	-	-
	O25	67.6	102.9	MK	-	-	-	-	-	-	-	-
	O27	65.8	95.9	105.3	MK	-	-	-	-	-	-	-
	O30	57.1	85.9	104	MK	-	-	-	-	-	-	-
	O32	61.5	96.3	119.4	89.5	MK	-	-	-	-	-	-
	O33	59.3	96	122.8	MK	-	-	-	-	-	-	-
	O34	56.9	91	115.9	MK	-	-	-	-	-	-	-
	O35	63.1	94.9	116.9	MK	-	-	-	-	-	-	-
	O37	62.4	93.7	MK	-	-	-	-	-	-	-	-
	O39	64.1	90.5	MK	-	-	-	-	-	-	-	-
	O40	63.1	91.8	102.3	MK	-	-	-	-	-	-	-

	O41	68.9	102.8	MK	-	-	-	-	-	-	-	-
	O44	64.6	98.9	MK	-	-	-	-	-	-	-	-
	O48	63.1	94.2	111.7	123	MK	-	-	-	-	-	-
	O50	49.7	82.6	117	MK	-	-	-	-	-	-	-
	O55	59.8	87.1	104.9	MK	-	-	-	-	-	-	-
	O57	64.6	96.2	MK	-	-	-	-	-	-	-	-
	O59	59.9	74.7	MK	-	-	-	-	-	-	-	-
	O62	60.6	92.3	106.5	109.3	MK	-	-	-	-	-	-
	O64	65.6	97.2	115.5	MK	-	-	-	-	-	-	-
	Mean	**62.4**	**93.4**	**111.5**	**114.5**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
	SD	**4.12**	**6.52**	**8.56**	**12.07**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

MK = moribund kill

Table E-3
10 Week Individual Body Mass (grams)
F1 Generation Female Quail

Dose Group	Animal ID	Day 0	Day 2	Day 9	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
	Y52	7.1	9.4	27.6	53.8	92.6	138.7	151.9	187	189.5	188.3	193.5	194
0 mg/kg	Y57	8.2	10.5	36.6	58.4	95.1	136.9	164.1	169.3	138.4	143.8	160.7	145.1
	Y64	8.3	10.7	33.2	53.4	88.1	131.4	157.6	171.4	175.5	176.9	186.8	192.4
	Y65	7.7	10.4	34.7	52.9	82.1	121.1	144.2	182	179.9	173.4	172.4	179.2
	Y67	6	8.8	28.6	46.6	80.5	119.1	138.8	181.8	175	157.4	175.9	177.9
	Y70	6.5	8.7	28.4	48.6	84.6	130.6	150.5	186.7	179.8	193.4	196.7	189.7
	Y73	7.8	9.5	30.8	52.4	88.9	127.9	144.3	176.3	188.9	196.3	195.6	193.5
	Y78	7.2	9.8	35.4	56.8	88.6	116.3	138.7	172.4	164.1	150.2	162.3	161.5
	Y80	8	7.8	31.5	50.2	84	120.4	142.8	182.9	181	154.1	160.8	152.9
	Y82	7.3	9.5	32.6	56.6	98.8	141.8	161.7	180.5	187	183.3	193.5	197.9
	Y83	6.5	11	32.7	54.1	91.7	127	146.2	160.7	184.1	180.8	176.8	176.3
	Y85	7.6	11.7	35.5	58.1	96.7	129.7	146.9	173.2	178.3	178.2	176.1	187.4
	Y86	7.2	8.7	28.3	50.2	92.8	127.1	145.9	174.7	170.4	151.2	164.8	170.7
	Mean	**7.3**	**9.7**	**32.0**	**53.2**	**89.6**	**128.3**	**148.7**	**176.8**	**176.3**	**171.3**	**178.1**	**178.3**
	SD	**0.70**	**1.10**	**3.08**	**3.65**	**5.69**	**7.79**	**8.11**	**7.56**	**13.48**	**17.83**	**13.77**	**16.75**
	P52	8.1	8.9	24.6	47.7	84.6	129.2	161.6	170.5	167.9	175.1	173.4	183.3
	P54	7.1	7.7	28.7	56.9	95.6	143.1	167.9	201.4	192.6	198.8	152.7	197.5
20 mg/kg	P57	7.6	9.6	27.5	53.1	91.8	139.6	147.9	172.4	176.2	177.3	177.2	169.5
	P58	8.3	10.1	29	51.2	89.3	129	155.6	179.2	171.9	149.2	154.1	158
	P60	7.2	11.4	32.5	54.7	87.6	129.9	143.1	178.3	172.8	175.2	176.7	160.7
	P61	8	11.4	32.7	52.5	90	133.5	161.5	188.4	183.7	167.3	186.3	189.4
	P62	8.2	10.9	30.5	51.7	92.3	135.2	152.8	183.5	182.3	184.1	184.5	198.1
	P65	7.5	11.8	35.7	57.3	94.3	136.2	155.7	182.3	195.8	181.8	168.8	185.3
	P70	7.3	11.8	34.2	59.1	96.4	130.8	147.1	179.9	179.3	177.3	173.5	178.7
	P73	7.8	10.6	34.5	60.2	99.9	137.4	163.6	159.9	170.3	175.6	178.2	174.5

	P77	8	11.3	34.6	60.1	98	140.4	165.4	201	196	196.8	183.1	173.1
	P78	8	11.8	36.7	58.3	97.7	132.3	162.6	167	163.1	174.9	170.6	177.2
	P86	7.3	11.2	33.9	57.1	100.2	128.9	147.7	177.2	203.4	189.1	179.8	192.9
	P89	8.3	11.2	33.2	56.8	96.2	138.2	164.2	191	206.9	195.1	201	210.4
	P92	7	10	31.4	54.7	93.2	134.2	151.4	183.6	181	182.3	161.3	181.1
	P94	7.1	10.1	35.8	64.2	109.6	153.4	169.5	213.4	210.9	221.2	226.4	224.7
	P95	7.9	11	30.9	50.7	88.4	125.2	145.4	174.3	192.5	189.8	189.3	195.8
	Mean	**7.7**	**10.6**	**32.1**	**55.7**	**94.4**	**135.1**	**156.6**	**182.5**	**185.1**	**183.0**	**178.6**	**185.3**
	SD	**1.70**	**11.15**	**12.46**	**20.26**	**21.50**	**13.17**	**16.98**	**13.44**	**14.33**	**15.47**	**17.33**	**17.10**
	G53	7.3	8.6	32.8	54.8	85.1	126.4	144.7	138.6	168.4	171.4	160.5	165.8
	G54	7.1	9.8	35.2	58.4	95.6	130.7	143.6	174.4	176.2	161.6	172.2	174.1
100 mg/kg	G59	8.4	11.2	34.6	53.9	90.9	129.5	154.9	193.7	187.9	178.2	186.1	181.6
	G63	7.3	9.8	36.4	57.8	93.1	129.5	148.2	173.8	167.1	167.4	181.8	182.8
	G69	6.8	8.5	28.8	47.2	84.1	125.5	138.8	167.6	181.3	181.7	177	186.8
	G71	7.9	11.2	39.1	57.9	96	132.5	128.1	163.9	184.5	176.2	196.7	179.3
	G72	9.4	11.8	38.6	59.4	98.4	136.2	145.4	164.1	177.5	181.7	173.6	171.2
	G75	8	11.3	22.4	38	75.8	119.9	148.9	178.3	181.8	187.9	173.1	174.6
	G77	8	11.2	29	44.8	79.1	119.1	141.7	166.7	192.3	192.2	189.5	191
	G78	7.9	10.8	29.7	47.9	85.3	121.7	149.1	175.8	177.8	179.2	181.2	188.3
	G79	7.8	10.6	34.4	56.2	95.1	130.5	154.6	187.3	192.4	178.6	202.2	199.6
	G80	8	10.9	36	58.7	97.9	133.7	140.7	168.5	186.2	176.3	195.4	198.8
	G82	9.7	13	40.1	68.1	109.1	141.3	158.5	193.9	204.2	199.2	202.4	198.6
	G84	8.2	13	33.2	55.6	88.8	130.6	144.4	169	186.8	186.3	178.2	183.9
	G88	9.3	11.7	36.6	60.4	99.6	127	150.2	182.7	213	184.1	190.7	199.5
	Mean	**8.1**	**10.9**	**33.8**	**54.6**	**91.6**	**128.9**	**146.1**	**173.2**	**185.2**	**180.1**	**184.0**	**185.1**
	SD	**0.81**	**1.27**	**4.54**	**7.15**	**8.40**	**5.79**	**7.21**	**13.76**	**12.19**	**9.44**	**12.11**	**10.97**

Table E-4
10 Week Individual Body Mass (grams)
F1 Generation Male Quail

Dose Group	Animal ID	Day 0	Day 2	Day 9	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
	Y51	8	8.8	30.2	49.9	80.7	115.3	131.2	138.9	145.8	143.5	147.5	149.7
0 mg/kg	Y53	7.9	8.3	29.2	53.4	91.9	127.2	135.7	144	148.9	155.4	160.2	160.3
	Y54	6.9	6.8	24.9	44.9	76.1	118.7	132	136.7	141.9	139.7	142.8	145.6
	Y58	6.5	7.2	27.7	48.9	85	122	134.3	134.3	139.3	135.4	139.7	143.3
	Y60	5.8	9	30.4	49.9	80.1	113.7	124.8	134.8	139.2	138.9	141.7	145.5
	Y63	7.5	9.1	28.4	46.8	82.4	121.1	138.9	147	150.2	136.2	150	150.4
	Y66	8.2	8.6	29.8	49.2	83	121.1	138	141.6	146.8	138.7	141.6	148.8
	Y69	8.4	9.3	31.9	52.9	94.3	129.8	136.3	139.5	144	149.3	146.8	149.5
	Y71	7.2	8.9	30.1	52.5	85.9	119.9	125.6	123.7	123.7	121.8	130	132.1
	Y72	7.6	9.3	33.9	57.7	92.4	125.4	136.1	142.8	146.8	149.8	150.9	151.4
	Y74	8.3	11.3	36.1	54.7	91.3	122.6	130.4	132.4	133.8	140.1	144.6	146.4
	Y81	8.1	11.9	41.8	67.9	110.3	149.7	148.3	155.1	161.7	171.3	175.6	178
	Y84	7.1	10.5	37.2	60.6	101.6	137	145.7	146.2	148.7	146.5	153.4	156.6
	Y87	7	8.9	28.9	52.5	92	124.8	138.1	146	150.2	154.1	154.8	156.4
	Y88	7.3	9.3	32.9	56.5	93.7	124.7	131.1	141.9	149.5	150	149.3	154.6
	Mean	**7.5**	**9.1**	**31.6**	**53.2**	**89.4**	**124.9**	**135.1**	**140.3**	**144.7**	**144.7**	**148.6**	**151.2**
	SD	**0.71**	**1.28**	**4.12**	**5.61**	**8.62**	**8.62**	**6.22**	**7.44**	**8.34**	**11.31**	**10.38**	**9.98**
	P51	8.6	9.6	27.8	54.4	91.2	136	153.6	160.6	169.9	169	171.4	172.5
	P59	7.7	11	29.7	51.7	89.3	123.7	131.2	146.7	149.8	149.3	150.7	150.5
20 mg/kg	P63	7.7	11	35.5	59.7	99.5	136.1	152.1	152.4	156.4	155.1	161	166.1
	P67	7.6	11.1	32.9	58.7	96.2	134.7	149.1	155.1	161.8	165.4	172.4	179.2
	P68	7.2	9.6	31.6	55.3	90.4	124.6	142.2	143.4	147.6	145.2	146.8	149.8
	P69	7.1	10.1	30.1	51.8	90.3	123.8	135.2	138.6	142.3	144.1	145.9	145.1
	P72	7.1	11.2	39.2	64.5	105.5	139.9	146.6	156.3	159.1	164.9	170.2	169.7
	P74	7.1	11.4	37.7	61	102.6	141.7	152.3	155.6	163.2	160.8	163.4	167.6

	P75	7.9	11	30.3	55	94.1	135.2	144.2	158.2	152.8	153.8	157.1	160
	P79	8.3	9.9	29.4	53	88	127.8	145.8	165.1	168.6	158.5	157.4	162.1
	P80	8	10.6	32.7	60.4	94.4	143.3	135.1	152.9	157.1	153.3	158.2	160.1
	P81	7.9	11.6	32.4	57.9	98	141.1	155.1	158.5	162.3	160	167.2	169.8
	P84	6.9	9.5	28.7	51.8	90.6	129.9	139.6	147	149.6	146.7	153.4	156.8
	P90	7.9	10.2	34.7	60	98.2	136.1	156.2	163.5	173.4	172.5	178	181.7
	P91	8.1	11.4	31.6	51	87.5	123.5	133.6	136.4	136	136.9	137.1	138.8
	P96	6.7	10.8	35.6	58.7	99.1	133.7	148.1	150	158.7	162	164.1	164.6
	P98	7.7	11.4	31.7	54.4	91.5	126.3	141.2	146.2	152.3	158.8	164.6	166.8
	P99	8.6	11.6	33.9	58.3	94.7	125.3	130.2	136.8	141.9	146	146.6	149.5
	Mean	**7.7**	**10.7**	**32.5**	**56.5**	**94.5**	**132.4**	**144.0**	**151.3**	**155.7**	**155.7**	**159.2**	**161.7**
	SD	**0.56**	**0.72**	**3.11**	**3.90**	**5.14**	**6.85**	**8.41**	**8.78**	**10.17**	**9.59**	**10.92**	**11.60**
	G52	6.1	8.7	30.1	51.6	90.3	125.3	143.4	141.6	146.9	154.1	157.1	161.3
	G55	7.1	8.9	31.6	51.8	90.3	126.7	140.6	147.5	145.2	149.1	154.1	161.3
100 mg/kg	G57	7.3	10.1	32	51.3	86.1	113.6	129	133.4	131.1	131	137.5	139.9
	G58	8.3	10.3	34.9	56.6	91.2	126.2	148	158.8	161.4	162.5	163	165.1
	G62	7.5	11.3	35.6	54.7	93.1	132.3	149.4	152.5	149.4	153.4	150.2	149.8
	G64	6.7	9.5	32.7	53.7	89.8	127.1	140.5	139.7	140	148.9	149	154.2
	G65	8.5	10.7	35	52.8	78.7	118.4	137.7	145.2	148.9	153.1	153.1	156
	G66	7	9.5	30.3	50.3	84	118.2	136.7	135.1	134.5	138.1	141	141.8
	G68	7	9.5	30.6	51.5	82.7	122.1	134	146.9	148.1	151.5	153.3	158.1
	G74	6.8	8.1	23.1	40.5	72.3	108	133.2	139.9	141.8	144	145.5	145.2
	G76	8.2	11.5	28	45.5	80.9	115.5	132.6	142.6	147.3	157.1	161.6	165.7
	G81	7.9	10.8	33.4	57.7	96.8	130.4	146.3	144.3	150.7	154	157	158
	G85	6	8.4	30.2	52.5	85.1	117.4	137.3	144.3	153.4	154	163.6	175.5
	G86	8.2	10.8	36.5	62	97.4	130.2	140.6	154.1	163.4	152.4	149.8	150.7
	G87	8	7.9	26.6	45.2	77.3	109.6	124.8	129.6	126.7	131.6	134.6	135.7
	G90	7.6	9.5	30.2	53.2	87.9	118.3	132.6	135.7	139.1	140.3	140.5	140.7
	Mean	**7.4**	**9.7**	**31.3**	**51.9**	**86.5**	**121.2**	**137.9**	**143.2**	**145.5**	**148.4**	**150.7**	**153.7**
	SD	**0.74**	**1.09**	**3.40**	**4.94**	**6.77**	**7.23**	**6.63**	**7.82**	**9.89**	**9.02**	**8.96**	**11.04**

Appendix F

Individual and Summary Male Developmental Data

Table F-1
12 Week Individual Cloaca Size (mm^2)
F0 Generation Male Quail

Dose Group	Animal ID	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
	Y2	120	255	288	342	420	380	400
0 mg/kg	Y7	132	306	323	360	400	360	360
	Y8	144	256	323	440	484	440	440
	Y10	144	255	289	380	462	400	400
	Y19	144	288	288	380	418	420	420
	Y21	120	196	MK	-	-	-	-
	Y26	156	306	360	440	484	440	440
	Y35	72	238	306	400	462	400	420
	Y38	99	224	288	380	418	420	420
	Y39	110	168	289	400	400	484	484
	Y42	120	256	324	380	380	400	400
	Y51	121	240	288	380	440	420	440
	Mean	**123.5**	**249.0**	**306.0**	**389.3**	**433.5**	**414.9**	**420.4**
	SD	**23.09**	**40.88**	**23.86**	**29.83**	**35.41**	**33.18**	**31.77**
	P3	132	306	323	420	420	420	420
20 mg/kg	P5	121	210	306	399	418	420	420
	P12	81	196	289	324	361	324	324
	P14	156	255	288	400	400	400	440
	P18	156	272	342	440	462	440	440
	P19	156	289	272	440	440	440	440
	P20	121	195	272	440	400	440	440
	P21	132	256	256	380	440	380	380
	P26	132	289	306	360	400	360	360
	P34	132	225	289	380	440	440	440
	P38	121	256	306	440	440	440	440
	P40	144	342	342	399	420	418	418
	Mean	**132.0**	**257.6**	**299.3**	**401.8**	**420.1**	**410.2**	**413.5**
	SD	**20.97**	**45.43**	**27.05**	**36.85**	**27.05**	**37.73**	**38.51**
	G2	121	272	360	418	440	418	440
100 mg/kg	G8	90	182	224	361	380	361	361
	G9	64	225	289	360	380	342	342
	G17	110	256	272	399	440	440	440
	G19	120	272	323	440	440	462	483
	G20	132	289	324	399	399	420	420
	G23	143	256	420	399	MK	-	-
	G24	132	225	360	380	380	440	440
	G27	120	210	306	440	440	440	440
	G28	132	256	324	399	420	440	440
	G32	72	182	210	361	361	400	420

	G39	90	256	306	380	380	380	380
	Mean	**110.5**	**240.1**	**309.8**	**394.7**	**405.5**	**413.0**	**418.7**
	SD	**25.62**	**35.10**	**57.98**	**28.01**	**30.98**	**37.94**	**41.37**

MK = moribund kill

Table F-2
10 Week Individual Cloaca Size (mm^2)
F1 Generation Male Quail

Group	Animal ID	Week 6	Week 7	Week 8	Week 9	Week 10
0 mg/kg	Y51	121	168	225	256	289
	Y53	121	196	225	324	361
	Y54	121	225	256	324	324
	Y58	144	210	289	324	361
	Y60	100	196	324	324	324
	Y63	121	195	256	324	324
	Y66	110	182	225	289	324
	Y69	121	169	289	324	400
	Y71	121	195	289	324	324
	Y72	144	225	324	324	400
	Y74	169	252	400	400	484
	Y81	144	288	324	400	400
	Y84	121	156	256	324	324
	Y87	100	144	256	289	324
	Y88	144	255	289	324	324
	Mean	**126.8**	**203.7**	**281.8**	**324.9**	**352.5**
	SD	**18.74**	**39.71**	**47.71**	**36.46**	**49.81**
20 mg/kg	P51	144	225	289	289	289
	P59	144	224	324	400	400
	P63	169	225	324	324	361
	P67	121	240	256	289	361
	P68	121	182	256	289	361
	P69	169	224	256	289	361
	P72	144	210	324	324	361
	P74	100	195	225	324	324
	P75	121	182	225	256	289
	P79	100	100	121	196	196
	P80	121	225	256	324	324
	P81	144	169	225	324	324
	P84	144	182	289	324	324
	P90	121	224	256	256	324
	P91	121	224	256	289	324
	P96	121	210	324	324	400
	P98	144	182	289	289	289
	P99	121	224	256	324	361
	Mean	**131.7**	**202.6**	**263.9**	**301.9**	**331.8**
	SD	**19.73**	**33.18**	**49.55**	**41.98**	**47.73**
100 mg/kg	G52	121	240	289	324	324
	G55	121	196	256	256	289
	G57	121	144	196	225	256

G58	121	156	196	256	324
G62	121	196	256	256	256
G64	121	156	196	225	256
G65	121	132	169	196	196
G66	144	169	225	225	289
G68	121	182	225	256	361
G74	100	156	196	256	289
G76	100	169	225	256	289
G81	144	210	324	324	324
G85	100	196	225	256	256
G86	121	182	256	256	289
G87	81	144	196	196	196
G90	100	168	196	196	225
Mean	**116.1**	**174.8**	**226.6**	**247.4**	**276.2**
SD	**16.41**	**28.10**	**40.76**	**38.09**	**46.18**

Appendix G

Individual and Summary Female Developmental Data

Table G-1
12 Week Individual Weekly Egg Production (number of eggs laid)
F0 Generation Female Quail

Group	Animal ID	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11
	Y1	0	1	5	7	6	7	6
	Y4	1	6	7	5	7	7	7
0 mg/kg	Y11	0	1	6	7	5	6	7
	Y22	0	0	0	0	0	6	6
	Y23	0	2	6	6	7	5	7
	Y24	0	0	0	0	0	6	7
	Y29	0	0	5	5	4	5	7
	Y30	0	0	3	5	6	6	4
	Y34	0	6	5	7	6	7	6
	Y46	0	0	5	6	5	6	7
	Y52	0	5	3	4	5	7	6
	Y59	0	6	6	7	7	7	MK
	Mean	**0.1**	**2.3**	**4.3**	**4.9**	**4.8**	**6.3**	**6.4**
	SD	**0.29**	**2.67**	**2.30**	**2.50**	**2.44**	**0.75**	**0.92**
	P1	0	5	4	6	7	6	7
	P9	0	6	7	7	6	7	7
20 mg/kg	P10	1	5	6	6	6	7	7
	P16	0	7	6	6	6	7	6
	P17	0	4	6	7	6	7	6
	P22	2	7	6	6	7	6	6
	P24	1	6	7	6	7	7	6
	P31	0	2	7	5	6	6	7
	P39	0	4	6	6	6	7	6
	P42	0	2	7	7	6	7	6
	Mean	**0.4**	**4.8**	**6.2**	**6.2**	**6.3**	**6.7**	**6.4**
	SD	**0.70**	**1.81**	**0.92**	**0.63**	**0.48**	**0.48**	**0.52**
	G7	0	2	6	7	7	7	7
	G11	0	2	6	6	7	6	7
100 mg/kg	G14	0	0	4	6	6	6	6
	G15	0	0	4	6	7	6	7
	G18	0	0	5	7	6	7	6
	G26	0	1	7	6	7	7	7
	G30	0	2	5	7	6	6	7
	G31	0	3	7	6	7	6	7
	G35*	0	2	7	7	5	6	6
	G38	1	7	6	6	7	6	7
	G40	2	7	6	7	7	7	6
	G42	0	2	7	6	7	7	7
	Mean	**0.3**	**2.3**	**5.8**	**6.4**	**6.6**	**6.4**	**6.7**

	SD	**0.62**	**2.39**	**1.11**	**0.51**	**0.67**	**0.51**	**0.49**

Table G-2
10 Week Individual Weekly Egg Production (number of eggs laid)
F1 Generation Female Quail

Group	Animal ID	Week 5	Week 6	Week 7	Week 8	Week 9
	Y52	0	4	4	6	5
	Y57	3	6	6	5	7
0 mg/kg	Y64	0	0	1	0	0
	Y65	0	3	6	7	6
	Y67	0	6	7	6	7
	Y70	1	7	6	7	7
	Y73	0	0	3	6	7
	Y78	0	5	5	4	5
	Y80	1	6	7	5	7
	Y82	0	6	7	6	7
	Y83	0	0	0	5	6
	Y85	0	6	6	7	6
	Y86	0	4	6	6	7
	Mean	**0.4**	**4.1**	**4.9**	**5.4**	**5.9**
	SD	**0.87**	**2.56**	**2.29**	**1.85**	**1.93**
	P52	1	7	6	7	6
	P54	0	7	6	7	2
20 mg/kg	P57	0	3	7	6	5
	P58	0	3	6	5	6
	P60	0	6	6	6	7
	P61	0	6	6	5	6
	P62	0	3	6	7	6
	P65	0	3	5	7	6
	P70	0	2	6	7	6
	P73	4	5	6	6	7
	P77	0	7	6	7	7
	P78	4	7	6	7	6
	P86	0	0	7	7	6
	P89	0	0	0	2	5
	P92	0	5	5	7	6
	P94	0	6	6	7	7
	P95	0	4	6	7	6
	Mean	**0.5**	**4.4**	**5.6**	**6.3**	**5.9**
	SD	**1.33**	**2.34**	**1.54**	**1.31**	**1.17**
	G53	0	0	1	7	6
	G54	0	5	6	7	6
100 mg/kg	G59	0	6	7	6	7

G63	0	7	6	6	7
G69	0	3	6	7	6
G71	0	1	7	6	7
G72	0	0	1	2	6
G75	0	1	7	7	7
G77	0	0	6	7	5
G78	0	5	6	6	6
G79	0	3	7	6	6
G80	0	3	6	6	7
G82	0	2	7	6	4
G84	0	2	7	6	6
G88	0	0	2	5	5
Mean	**0.0**	**2.5**	**5.5**	**6.0**	**6.1**
SD	**0.00**	**2.33**	**2.20**	**1.25**	**0.88**

Appendix H

Individual and Summary Male Copulatory Behavior

Table H-1
12 Week Individual Copulatory Behavior at Week 7 (seconds & (number of attempts))
F0 Generation Male Quail

Dose Group	Animal ID	Mate ID	Day 1 Mount Lag	Day 1 Success Lag	Day 1 Copulations (Attempts)	Day 2 Mount Lag	Day 2 Success Lag	Day 2 Copulations (Attempts)	Day 3 Mount Lag	Day 3 Success Lag	Day 3 Copulations (Attempts)
	Y2	Y29	22	27	1 (1)	9	21	2 (2)	2	6	2 (2)
	Y7	Y30	170	179	1 (1)	4	6	2 (3)	9	12	1 (3)
0 mg/kg	Y8	Y23	30	33	2 (3)	7	36	1 (2)	6	9	1 (1)
	Y10	Y34	6	15	2 (3)	3	6	2 (2)	2	8	3 (3)
	Y19	Y59	19	23	1 (2)	5	8	1 (1)	10	30	2 (2)
	Y21	Y11	30	0	0 (2)	5	43	1 (3)	18	0	0 (2)
	Y26	Y46	10	14	2 (2)	4	9	2 (2)	6	9	2 (2)
	Y35	Y22	76	0	0 (4)	5	35	2 (3)	2	27	4 (7)
	Y38	Y52	23	30	2 (3)	5	69	1 (1)	8	12	3 (4)
	Y39	Y24	28	0	0 (3)	3	9	1 (1)	3	21	1 (2)
	Y42	Y4	5	97	1 (4)	2	43	2 (5)	3	15	4 (4)
	Y51	Y1	82	0	0 (3)	35	48	1 (3)	2	27	1 (2)
		Mean	**41.8**	**34.8**	**1.0 (2.6)**	**7.3**	**27.8**	**1.5 (2.3)**	**5.9**	**14.7**	**2.0 (2.8)**
		SD	**47.3**	**52.7**	**0.9 (1.0)**	**8.9**	**20.9**	**0.5 (1.2)**	**4.8**	**9.5**	**1.3 (1.6)**
	P3	P17	5	7	5 (8)	2	0	0 (5)	2	175	1 (6)
	P5	P22	108	0	0 (1)	19	35	1 (3)	7	9	1 (2)
20 mg/kg	P12	P15	149	155	1 (1)	16	20	1 (5)	4	0	0 (7)
	P14	P16	12	15	3 (3)	3	17	3 (5)	4	19	3 (5)
	P18	P10	39	0	0 (1)	9	12	1 (1)	3	19	1 (1)
	P19	P39	3	9	2 (2)	2	5	2 (6)	4	7	2 (2)
	P20	P1	48	57	2 (5)	3	28	2 (6)	4	0	0 (5)
	P21	P24	9	11	1 (1)	2	5	1 (1)	4	21	1 (2)
	P26	P42	12	14	1 (1)	2	12	2 (2)	2	18	2 (2)
	P34	P9	12	18	2 (3)	4	7	3 (3)	7	40	2 (5)
	P40	P31	14	43	1 (4)	6	0	0 (3)	7	10	3 (3)
		Mean	**37.4**	**29.9**	**1.6 (2.7)**	**6.2**	**12.8**	**1.5 (3.6)**	**4.4**	**28.9**	**1.5 (3.6)**
		SD	**48.0**	**45.0**	**1.4 (2.2)**	**6.0**	**11.3**	**1.0 (1.9)**	**1.9**	**49.7**	**1.0 (2.0)**
	G2	G11	9	14	2 (2)	4	60	1 (8)	4	6	2 (6)
	G8	G30	161	165	1 (1)	10	12	1 (3)	5	8	2 (3)

100 mg/kg	G9	G7	5	13	1 (8)	4	90	2 (6)	2	41	1 (4)
	G17	G42	35	38	1 (3)	12	17	3 (8)	6	8	4 (4)
	G19	G15	7	12	3 (5)	4	0	0 (6)	10	13	1 (2)
	G20	G26	3	10	1 (1)	6	14	1 (2)	3	8	1 (1)
	G23	G35	7	9	2 (2)	9	11	2 (2)	2	4	2 (3)
	G24	G18	11	0	0 (10)	4	0	0 (11)	2	13	2 (3)
	G27	G14	4	139	1 (4)	3	120	1 (5)	2	0	0 (6)
	G28	G38	7	33	2 (5)	11	14	1 (2)	8	0	0 (2)
	G32	G31	0	0	0 (0)	0	0	0 (0)	0	0	0 (0)
	G39	G40	14	140	1 (4)	14	18	2 (5)	5	7	1 (4)
		Mean	**21.9**	**47.8**	**1.3 (3.8)**	**6.8**	**29.7**	**1.2 (4.8)**	**4.1**	**9.0**	**1.3 (3.2)**
		SD	**44.7**	**61.8**	**0.9 (3.0)**	**4.3**	**39.1**	**0.9 (3.2)**	**2.9**	**11.0**	**1.2 (1.8)**

Table H-2
10 Week Individual Copulatory Behavior at Week 7 (seconds & (number of attempts))
F1 Generation Male Quail

Dose Group	Animal ID	Mate ID	Day 1 Mount Lag	Day 1 Success Lag	Day 1 Copulations (Attempts)	Day 2 Mount Lag	Day 2 Success Lag	Day 2 Copulations (Attempts)	Day 3 Mount Lag	Day 3 Success Lag	Day 3 Copulations (Attempts)
0 mg/kg	Y51	Y85	9	68	1 (3)	4	12	5 (7)	4	10	4 (4)
	Y53	Y83	10	13	1 (1)	4	6	1 (1)	4	7	1 (1)
	Y54	Y64	86	0	0 (3)	16	0	0 (4)	3	0	0 (4)
	Y58	Y67	21	64	2 (4)	3	60	1 (2)	3	20	1 (3)
	Y60	Y86	7	10	2 (3)	5	7	3 (5)	4	0	0 (4)
	Y63	Y52	27	0	0 (1)	6	8	3 (4)	5	8	2 (2)
	Y66	Y70	18	0	0 (2)	3	0	0 (4)	2	5	2 (3)
	Y69	Y59	6	36	3 (4)	3	43	4 (6)	2	18	3 (4)
	Y71	Y57	14	0	0 (4)	5	8	1 (2)	3	5	1 (2)
	Y72	Y65	8	11	3 (5)	3	5	2 (2)	5	7	3 (3)
	Y74	Y78	16	29	2 (3)	3	12	1 (2)	2	10	3 (3)
	Y84	Y80	18	20	2 (2)	3	20	2 (4)	3	11	3 (8)
	Y87	Y73	90	93	1 (1)	17	19	1 (1)	41	0	0 (1)
	Y88	Y82	7	9	2 (2)	7	17	2 (3)	5	7	2 (4)
		Mean	**24.1**	**25.2**	**1.4 (2.7)**	**5.9**	**15.5**	**1.9 (3.4)**	**6.1**	**7.7**	**1.8 (3.3)**
		SD	**27.8**	**29.7**	**1.1 (1.4)**	**4.7**	**16.8**	**1.5 (1.8)**	**10.1**	**6.0**	**1.3 (1.7)**
20 mg/kg	P51	P94	6	10	2 (3)	2	14	1 (4)	22	0	0 (3)
	P59	P57	41	0	0 (3)	8	10	1 (5)	3	11	1 (3)
	P63	P62	40	126	1 (2)	60	0	0 (1)	3	0	0 (1)
	P67	P65	6	9	1 (1)	6	8	1 (1)	3	6	2 (2)
	P68	P58	57	62	1 (1)	13	16	1 (1)	4	6	1 (2)
	P69	P73	6	90	1 (5)	9	0	0 (2)	9	12	3 (3)
	P72	P54	5	17	1 (3)	5	7	3 (4)	3	0	0 (4)
	P74	P86	6	9	2 (2)	3	20	1 (2)	3	5	2 (3)
	P75	P92	0	0	0 (0)	0	0	0 (0)	30	0	0 (3)
	P79	P89	31	0	0 (3)	14	0	0 (2)	10	0	0 (3)
	P80	P61	23	28	2 (2)	5	7	2 (2)	3	41	1 (4)
	P81	P77	51	0	0 (3)	19	27	1 (1)	0	0	0 (0)
	P84	P60	32	37	1 (1)	7	42	1 (7)	5	7	2 (2)
	P91	P78	31	35	1 (1)	3	5	2 (3)	7	11	2 (5)

	P96	P95	11	19	2 (2)	4	11	2 (4)	4	36	1 (3)
	P98	P70	29	0	0 (10)	12	14	3 (4)	3	11	4 (4)
	P99	P52	16	25	1 (1)	6	48	1 (5)	7	0	0 (5)
		Mean	**23.0**	**27.5**	**0.9 (2.5)**	**10.4**	**13.5**	**1.2 (2.8)**	**7.0**	**8.6**	**1.1 (2.9)**
		SD	**17.7**	**35.3**	**0.7 (2.3)**	**13.7**	**14.1**	**1.0 (1.9)**	**7.7**	**12.2**	**1.2 (1.3)**
	G52	G71	6	9	1 (3)	2	6	3 (4)	8	10	3 (4)
	G55	G75	31	0	0 (3)	15	33	1 (2)	4	8	1 (1)
100 mg/kg	G57	G53	0	0	0 (0)	16	0	0 (9)	11	0	0 (10)
	G58	G88	34	0	0 (3)	7	87	1 (4)	3	11	3 (3)
	G62	G77	13	29	1 (2)	5	7	2 (3)	2	4	2 (2)
	G64	G78	19	0	0 (4)	2	23	1 (5)	5	0	0 (4)
	G65	G59	53	72	2 (4)	13	16	2 (2)	2	11	2 (3)
	G66	G54	10	0	0 (4)	11	29	1 (3)	27	0	0 (2)
	G68	G84	0	0	0 (0)	0	0	0 (0)	0	0	0 (0)
	G74	G69	18	0	0 (3)	7	0	0 (5)	4	7	2 (4)
	G76	G80	6	0	0 (9)	9	33	1 (7)	12	148	1 (8)
	G81	G79	5	8	3 (3)	2	7	3 (6)	2	4	3 (6)
	G85	G82	12	15	1 (3)	7	10	1 (4)	2	26	1 (5)
	G87	G63	32	35	2 (2)	4	16	2 (6)	3	11	5 (9)
	G90	G72	57	120	1 (5)	2	45	2 (4)	5	8	4 (4)
		Mean	**19.7**	**19.2**	**0.7 (3.2)**	**6.8**	**20.8**	**1.3 (4.3)**	**6.0**	**16.5**	**1.8 (4.3)**
		SD	**18.0**	**34.3**	**1.0 (2.1)**	**5.1**	**23.0**	**1.0 (2.2)**	**6.7**	**37.0**	**1.6 (2.9)**

Appendix I

Individual and Summary Fertility and Offspring Data

Table I-1
12 Week Individual Egg Fertility (number of eggs)
F0 Generation Female Quail

Dose Group	Animal ID	Fertile	Total	Percent Fertility
	Y1	5	6	83.3
	Y4	4	6	66.7
0 mg/kg	Y22	5	5	100.0
	Y23	4	5	80.0
	Y24	6	6	100.0
	Y29	6	6	100.0
	Y30	4	5	80.0
	Y34	6	6	100.0
	Y46	5	5	100.0
	Y52	4	5	80.0
			Mean	**89.0**
			SD	**12.4**
	P1	5	6	83.3
	P9	6	6	100.0
20 mg/kg	P10	6	6	100.0
	P16	6	6	100.0
	P17	5	6	83.3
	P22	6	6	100.0
	P24	6	6	100.0
	P31	6	6	100.0
	P39	6	6	100.0
	P42	6	6	100.0
			Mean	**96.7**
			SD	**7.0**
	G7	4	5	80.0
	G11	5	5	100.0
100 mg/kg	G14	5	5	100.0
	G15	0	5	0.0
	G18	5	5	100.0
	G26	5	5	100.0
	G30	5	5	100.0
	G31	4	5	80.0
	G38	5	5	100.0
	G40	5	5	100.0
	G42	5	5	100.0
			Mean	**87.3**
			SD	**30.0**

Table I-2
10 Week Individual Egg Fertility (number of eggs)
F1 Generation Female Quail

Dose Group	Animal ID	Fertile	Total	Percent Fertility
	Y52	4	4	100.0
	Y57	3	3	100.0
0 mg/kg	Y65	4	4	100.0
	Y67	4	4	100.0
	Y70	3	4	75.0
	Y73	4	4	100.0
	Y78	3	3	100.0
	Y80	4	4	100.0
	Y82	4	4	100.0
	Y83	4	4	100.0
	Y85	4	4	100.0
	Y86	4	4	100.0
			Mean	**97.9**
			SD	**7.2**
	P52	4	4	100.0
	P54	2	2	100.0
20 mg/kg	P57	4	4	100.0
	P58	3	3	100.0
	P60	4	4	100.0
	P61	4	4	100.0
	P62	4	4	100.0
	P65	3	4	75.0
	P70	4	4	100.0
	P73	4	4	100.0
	P77	4	4	100.0
	P78	4	4	100.0
	P80	0	1	0.0
	P86	4	4	100.0
	P89	4	4	100.0
	P92	3	3	100.0
	P94	4	4	100.0
	P95	4	4	100.0
			Mean	**93.1**
			SD	**24.0**
	G53	4	4	100.0
	G54	2	3	66.7
100 mg/kg	G59	3	3	100.0
	G63	4	4	100.0

G69	4	4	100.0
G71	4	4	100.0
G72	3	3	100.0
G75	4	4	100.0
G77	4	4	100.0
G78	3	4	75.0
G79	3	4	75.0
G80	4	4	100.0
G82	4	4	100.0
G84	2	4	50.0
G88	4	4	100.0
		Mean	**91.1**
		SD	**16.2**

Table I-3
10 Week Individual Offspring Data
F0 Generation Female Quail

Group	Animal ID	Egg 1	Egg 2	Egg 3	Egg 4	Egg 5	Egg 6
	Y1	Y86	Y87	U	U	U	U/I
0 mg/kg	Y4	Y76*	Y84	U	U/I	U/I	U
	Y22	Y55*	Y60	Y67	Y80	U	-
	Y23	Y63	Y73	Y83	U	U/I	-
	Y24	Y54	Y58	Y59	Y61	Y70	Y77
	Y29	Y51	Y57	Y64	Y75*	Y78	Y79*
	Y30	Y56*	Y65	Y71	Y85	U/I	-
	Y34	Y52	Y53	Y72	Y82	Y68*	Y88
	Y46	Y66	Y69	Y74	Y81	U	-
	Y52	Y62*	U	U	U	U/I	-
	P1	P54	P72	P74	P94	P96	U/I
	P9	P53*	P56*	P64*	P71*	U	U
20 mg/kg	P10	P66*	P67	P78	P97*	P98	U
	P16	P61	P62	P63	P73	P75	P95
	P17	P51	P79	P89	P93*	P99	U/I
	P22	58	81	U	U	U	U
	P24	P52	P76*	P83*	P87*	P91	U
	P31	P55*	P59	P60	P65	P70	P86
	P39	P57	P69	P84	P85*	P88*	P92
	P42	P68	P77	P80	P82*	P90	U
	G7	G64	G74	U	U/I	U	-
	G11	G58	G65	G70*	G77	G84	-
100 mg/kg	G14	G87	G89*	G90	U	U	-
	G15	U/I	U/I	U/I	U/I	U/I	-
	G18	G52	G54	G56*	G66	G69	-
	G26	G67*	G75	G76	U	U	-
	G30	G59	G61*	G62	G71	U	-
	G31	G72	G82	G88	U/I	U	-
	G38	G55	G57	G63	G73*	G85	-
	G40	G51*	G53	G60*	G68	G83*	-
	G42	G78	G79	G80	G81	G86	-

*Animal not used in study; U = unhatched egg; U/I = unhatched, infertile egg

Appendix J

Individual and Summary Eggshell Strength Data

Table J-1
12 Week Individual Eggshell Strength (kg)
F0 Generation Female Quail

Group	Animal ID	Egg 1	Egg 2	Egg 3	Egg 4	Egg 5	Egg 6	Egg 7	Average
	Y1	1.071	0.966	1.029	0.95	-	-	-	1.004
	Y4	1.064	1.357	1.215	1.459	1.796	-	-	1.378
0 mg/kg	Y11	1.463	1.242	1.309	1.264	1.152	1.369	-	1.300
	Y22	1.292	1.224	1.087	0.362	-	-	-	0.991
	Y23	1.413	1.413	1.201	1.224	1.418	1.54	-	1.368
	Y24	1.042	1.241	1.108	1.215	1.113	-	-	1.144
	Y29	1.522	1.376	1.541	1.803	-	-	-	1.561
	Y30	0.574	1.123	-	-	-	-	-	0.849
	Y34	0.786	0.806	0.736	0.722	0.789	-	-	0.768
	Y46	1.033	1.021	0.949	0.916	1.075	-	-	0.999
	Y52	0.987	0.826	0.681	0.895	0.705	-	-	0.819
	Y59	1.07	1.138	-	-	-	-	-	1.104
								Mean	**1.107**
								SD	**0.250**
	P1	1.124	1.14	1.156	1.387	1.576	-	-	1.277
	P9	1.237	1.348	1.247	0.963	-	-	-	1.199
20 mg/kg	P10	1.276	1.454	1.349	1.289	-	-	-	1.342
	P16	1.028		1.022	0.894	-	-	-	0.981
	P17	1.522	1.303	1.183	-	-	-	-	1.336
	P22	1.239	1.181	-	-	-	-	-	1.210
	P24	1.127	1.233	0.94	1.184	-	-	-	1.121
	P31	1.083	1.104	-	-	-	-	-	1.094
	P39	1.405	1.485	1.391	-	-	-	-	1.427
	P42	1.41	1.129	1.572	-	-	-	-	1.370
								Mean	**1.236**
								SD	**0.141**
	G7	0.941	1.068	0.92	1.142	0.486	1.21	-	0.961
	G11	1.143	1.177	1.356	1.165	1.394	1.315	-	1.258
100 mg/kg	G14	1.399	1.111	1.596	1.337	1.241	-	-	1.337
	G15	1.363	1.227	1.305	1.229	1.178	0.831	-	1.189
	G18	0.942	1.012	1.009	1.109	1.059	-	-	1.026
	G26	1.261	1.303	1.28	1.117	1.146	-	-	1.221
	G30	1.147	0.516	0.473	1.167	0.776	-	-	0.816
	G31	1.286	1.194	1.127	0.967	1.143	0.759	-	1.079
	G35*	1.069	0.757	0.947	0.829	0.942	-	-	0.909
	G38	1.75	1.44	1.222	1.339	1.441	1.616	1.092	1.414
	G40	1.456	1.234	1.225	0.923	1.257	1.355	-	1.242
	G42	1.306	1.352	1.163	1.458	1.284	1.852	-	1.403
								Mean	**1.155**
								SD	**0.196**

Table J-2
10 Week Individual Egg Strength (kg)
F1 Generation Female Quail

Group	Animal ID	Egg 1	Egg 2	Egg3	Egg 4	Average
	Y52	1.468	0.965	-	-	1.217
	Y57	1.097	-	-	-	1.097
0 mg/kg	Y64	1.484	1.778	1.626	-	1.629
	Y65	1.282	0.478	-	-	0.880
	Y67	1.493	1.475	1.492	-	1.487
	Y70	1.153	0.893	0.970	0.960	0.994
	Y73	1.356	-	-	-	1.356
	Y78	1.384	1.484	1.210	-	1.359
	Y80	1.492	1.298	1.442	-	1.411
	Y82	0.377	-	-	-	0.377
	Y83	1.451	1.345	1.607	-	1.468
	Y85	1.163	1.097	-	-	1.130
	Y86	1.468	0.965	-	-	1.217
					Mean	**1.200**
					SD	**0.340**
	P52	1.557	1.695	-	-	1.626
	P54	1.371	1.698	-	-	1.535
20 mg/kg	P57	1.571	1.222	-	-	1.397
	P60	1.381	1.153	1.440	-	1.325
	P61	1.217	1.131	1.378	-	1.242
	P62	1.147	1.118	-	-	1.133
	P65	0.900	1.194	0.914	-	1.003
	P70	1.175	1.096	1.183	-	1.151
	P73	1.329	1.176	-	-	1.253
	P77	1.074	1.153	1.134	-	1.120
	P78	0.942	1.120	-	-	1.031
	P86	1.304	1.415	1.470	-	1.396
	P89	0.641	1.027	-	-	0.834
	P92	1.579	1.663	1.870	-	1.704
	P94	0.956	1.204	1.216	-	1.125
	P95	1.147	1.339	1.286	-	1.257
					Mean	**1.258**
					SD	**0.233**
	G53	0.804	1.065	-	-	0.935
	G54	1.472	0.633	1.474	-	1.193
100 mg/kg	G59	1.183	0.935	1.396	1.091	1.151
	G63	1.205	1.291	1.146	-	1.214
	G69	1.134	1.161	1.259	-	1.185
	G71	1.351	1.432	1.440	-	1.408

G72	1.026	0.892	-	-	0.959
G75	0.941	0.830	-	-	0.886
G78	1.366	1.406	1.368	-	1.380
G79	1.471	1.539	1.245	-	1.418
G80	1.163	1.209	1.331	1.230	1.233
G82	1.091	1.126	-	-	1.109
G84	0.688	-	-	-	0.688
G88	1.128	1.095	1.151	-	1.125
				Mean	**1.134**
				SD	**0.210**

Appendix K

Individual and Summary Eggshell Thickness Data

Table K-1
12 Week Individual Eggshell Thickness (mm)
F0 Generation Female Quail

Group	Animal ID	Egg 1	Egg 2	Egg 3	Egg 4	Egg 5	Egg 6	Egg 7	Average
	Y1	0.215	0.228	0.199	0.229	-	-	-	0.218
	Y4	0.248	0.224	0.232	0.222	0.233	-	-	0.232
0 mg/kg	Y11	0.236	0.231	0.231	0.235	0.247	0.232	-	0.235
	Y22	0.233	0.241	0.208	0.227	-	-	-	0.227
	Y23	0.236	0.218	0.225	0.235	0.209	0.217	-	0.223
	Y24	0.203	0.195	-	-	-	-	-	0.199
	Y29	0.248	0.216	0.212	0.255	-	-	-	0.233
	Y30	0.189	0.207	-	-	-	-	-	0.198
	Y34	0.225	0.210	0.239	0.205	0.197	-	-	0.215
	Y46	0.223	0.231	0.935	0.209	0.198	-	-	0.359
	Y52	0.191	0.222	0.219	0.199	0.218	-	-	0.210
	Y59	0.240	0.235	0.199	0.229	-	-	-	0.237
								Mean	**0.232**
								SD	**0.042**
	P1	0.221	0.214	0.215	0.211	0.210	-	-	0.214
	P9	0.218	0.212	0.200	0.226	-	-	-	0.214
20 mg/kg	P10	0.244	0.228	0.230	0.203	-	-	-	0.226
	P16	0.210	0.199	0.204	0.211	-	-	-	0.206
	P17	0.237	0.216	0.211	-	-	-	-	0.221
	P22	0.225	0.240	-	-	-	-	-	0.233
	P24	0.234	0.225	0.241	0.224	-	-	-	0.231
	P31	0.214	0.213	-	-	-	-	-	0.214
	P39	0.248	0.255	0.257	-	-	-	-	0.253
	P42	0.243	0.252	0.250	-	-	-	-	0.248
								Mean	**0.226**
								SD	**0.015**

	G7	0.202	0.214	0.208	0.207	0.201	0.203	-	0.206
	G11	0.213	0.222	0.226	0.231	0.222	0.216	-	0.222
100 mg/kg	G14	0.215	0.226	0.218	0.228	0.229	-	-	0.223
	G15	0.230	0.211	0.225	0.208	0.239	0.210	-	0.220
	G18	0.199	0.209	0.201	0.217	0.209	-	-	0.207
	G26	0.247	0.242	0.267	0.240	0.236	-	-	0.246
	G30	0.203	0.234	0.198	0.205	0.205	-	-	0.209
	G31	0.221	0.213	0.215	0.233	0.239	0.242	-	0.227
	G35*	0.209	0.214	0.199	0.215	0.213	-	-	0.210
	G38	0.224	0.222	0.224	0.213	0.214	0.227	0.234	0.223
	G40	0.231	0.222	0.229	0.225	0.229	0.214	-	0.225
	G42	0.219	0.221	0.211	0.216	0.214	0.226	-	0.218
								Mean	**0.220**
								SD	**0.011**

Table K-2
10 Week Individual Eggshell Thickness (mm)
F1 Generation Female Quail

Group	Animal ID	Egg 1	Egg 2	Egg3	Egg 4	Average
	Y52	0.232	0.220	-	-	0.226
	Y57	0.213	-	-	-	0.213
0 mg/kg	Y65	0.232	0.284	0.250	-	0.255
	Y67	0.221	0.200	-	-	0.211
	Y70	0.216	0.198	0.205	-	0.207
	Y73	0.177	0.182	0.199	0.202	0.190
	Y78	0.203	-	-	-	0.203
	Y80	0.210	0.212	0.212	-	0.211
	Y82	0.241	0.227	0.222	-	0.230
	Y83	0.165	-	-	-	0.165
	Y85	0.230	0.236	0.227	-	0.231
	Y86	0.224	0.217	-	-	0.221
					Mean	**0.213**
					SD	**0.007**
	P52	0.210	0.221	-	-	0.216
	P54	0.253	0.261	-	-	0.257
20 mg/kg	P57	0.238	0.226	-	-	0.232
	P58	0.178	0.179	-	-	0.179
	P60	0.221	0.222	0.218	-	0.220
	P61	0.194	0.194	0.194	-	0.194
	P62	0.198	0.207	-	-	0.202
	P65	0.231	0.224	0.207	-	0.221
	P70	0.221	0.220	0.237	-	0.226
	P73	0.222	0.231	-	-	0.226
	P77	0.217	0.207	0.207	-	0.210
	P78	0.173	0.182	-	-	0.177
	P86	0.234	0.243	0.242	-	0.240
	P89	0.230	0.217	-	-	0.224
	P92	0.231	0.232	0.227	-	0.230
	P94	0.224	0.235	0.235	-	0.231
	P95	0.246	0.236	0.235	-	0.239
					Mean	**0.219**
					SD	**0.003**
	G53	0.210	0.202	-	-	0.206
	G54	0.201	0.197	0.203	-	0.200
100 mg/kg	G59	0.230	0.214	0.228	0.221	0.223
	G63	0.205	0.207	0.218	-	0.210
	G69	0.217	0.224	0.220	-	0.220
	G71	0.228	0.205	0.235	-	0.223

G72	0.186	0.185	-	-	0.186
G75	0.184	0.204	0.194	-	0.194
G77	0.203	0.188	0.209	-	0.200
G78	0.232	0.226	0.225	-	0.228
G79	0.246	0.245	0.236	-	0.242
G80	0.229	0.226	0.214	0.215	0.221
G82	0.200	0.198	-	-	0.199
G84	0.185	0.201	0.183	-	0.190
G88	0.244	0.222	0.227	-	0.231
				Mean	**0.211**
				SD	**0.004**

Appendix L

Individual and Summary Organ Mass Data

Table L-1
12 Week Individual Organ Mass
F0 Generation Female Quail

ABSOLUTE ORGAN MASS (GRAMS)

Group	Animal ID	Brain	Bursa	Heart	Liver	Ovaries	Oviduct	Spleen	Thyroid
	Y1	0.905	0.061	1.427	5.160	6.512	6.956	0.069	0.017
	Y4	0.762	0.022	1.746	5.372	8.583	7.879	0.058	0.018
	Y11	0.767	0.065	1.739	5.936	4.739	6.746	0.058	0.006
	Y22	0.791	0.021	1.433	4.756	5.846	5.894	0.061	0.016
0 mg/kg	Y23	0.861	0.019	1.567	8.236	5.702	5.583	0.090	0.013
	Y24	0.833	0.087	1.548	4.465	4.822	6.049	0.029	0.016
	Y29	0.763	ND	1.320	5.243	3.770	7.932	0.084	0.010
	Y30	0.859	0.041	1.151	4.560	7.847	6.634	0.050	0.010
	Y34	0.922	0.024	1.806	5.544	5.375	7.175	0.071	0.015
	Y46	0.878	0.018	1.307	5.800	6.891	6.834	0.054	0.012
	Y52	0.791	0.026	1.111	5.428	5.122	6.394	0.048	0.012
	Mean	**0.830**	**0.106**	**1.469**	**5.500**	**5.928**	**6.734**	**0.061**	**0.013**
	SD	**0.059**	**0.226**	**0.237**	**1.024**	**1.425**	**0.750**	**0.017**	**0.004**
	P1	0.813	0.040	1.940	4.707	6.177	5.791	0.117	0.011
	P9	0.791	0.036	1.690	5.206	4.991	6.462	0.049	0.022
	P10	0.826	0.040	1.688	6.089	8.552	8.853	0.070	0.013
	P16	0.724	0.043	1.450	4.869	5.674	6.345	0.061	0.007
20 mg/kg	P17	0.850	0.102	2.045	6.340	5.478	7.499	0.082	0.015
	P22	0.804	0.102	1.482	6.086	5.965	6.904	0.040	0.014
	P24	0.842	0.037	1.447	5.346	5.234	6.843	0.056	0.016

	P31	0.783	0.055	1.469	4.892	7.200	7.123	0.109	0.012
	P39	0.758	0.021	1.567	5.869	5.571	6.927	0.061	ND
	P42	0.827	0.130	1.609	6.016	6.267	8.113	0.074	0.012
	Mean	**0.802**	**0.061**	**1.639**	**5.542**	**6.111**	**7.086**	**0.072**	**0.013**
	SD	**0.039**	**0.037**	**0.209**	**0.604**	**1.059**	**0.887**	**0.025**	**0.004**
	G7	0.772	0.054	1.334	4.674	6.352	6.429	0.060	0.020
	G11	0.797	0.016	1.859	7.066	9.022	6.639	0.044	0.019
	G14	0.857	0.037	1.383	6.659	6.516	7.203	0.109	0.013
	G15	0.872	0.091	1.554	5.473	7.355	6.852	0.038	0.029
100 mg/kg	G18	0.753	0.051	1.490	4.658	4.973	6.820	0.029	0.006
	G26	0.763	0.054	1.655	6.487	8.554	7.730	0.079	0.021
	G30	0.799	0.036	1.666	5.032	4.646	6.388	ND	0.015
	G31	0.841	0.043	1.592	5.743	6.895	7.744	0.077	0.008
	G35	0.803	0.033	1.210	5.384	5.735	6.949	0.062	0.017
	G38	0.797	0.068	1.549	5.887	4.647	5.637	0.090	0.015
	G40	0.821	0.077	1.881	5.284	5.708	7.113	0.093	0.009
	G42	0.757	0.036	2.316	6.139	7.587	6.764	0.080	0.016
	Mean	**0.803**	**0.050**	**1.624**	**5.707**	**6.499**	**6.856**	**0.069**	**0.016**
	SD	**0.039**	**0.021**	**0.293**	**0.771**	**1.449**	**0.579**	**0.025**	**0.006**

ND = no data

Table L-2
12 Week Individual Organ Mass
F0 Generation Male Quail

ABSOLUTE ORGAN MASS (GRAMS)

Group	Animal ID	Brain	Bursa	Epididymis	Heart	Liver	Spleen	Testes	Thyroid
	Y2	0.697	0.108	0.106	1.413	2.972	0.030	3.890	0.008
	Y7	0.798	0.065	0.157	1.412	2.520	0.028	5.224	0.010
	Y8	0.815	0.106	0.150	1.475	3.130	0.040	4.926	0.012
	Y10	0.787	0.050	0.126	1.614	2.813	0.057	4.703	0.008
0 mg/kg	Y19	0.808	0.044	0.187	1.578	3.063	0.046	4.281	0.013
	Y26	0.764	0.084	0.217	1.476	2.737	0.058	5.610	0.023
	Y35	0.751	0.063	0.161	1.527	2.554	0.029	5.551	0.003
	Y38	0.702	0.058	0.142	1.605	3.247	0.027	5.424	0.008
	Y39	0.848	0.090	0.133	1.593	2.644	0.046	4.298	0.002
	Y42	0.802	0.040	0.143	1.469	2.599	0.038	4.290	0.008
	Y51	0.773	0.110	0.147	1.673	2.377	0.079	4.889	0.017
	Mean	**0.777**	**0.074**	**0.152**	**1.530**	**2.787**	**0.043**	**4.826**	**0.010**
	SD	**0.046**	**0.026**	**0.030**	**0.088**	**0.281**	**0.016**	**0.585**	**0.006**
	P3	0.764	0.074	0.201	1.671	2.873	0.037	5.005	0.006
	P5	0.777	0.035	0.131	1.542	3.073	0.032	4.940	0.008
	P12	0.770	0.087	0.113	1.869	2.833	0.066	4.433	0.008
	P14	0.815	0.094	0.150	1.509	2.804	0.025	4.014	0.008
	P18	0.703	0.109	0.142	1.747	3.004	0.045	3.716	0.010
	P19	0.805	0.041	0.126	1.591	3.133	0.046	5.058	0.014
20 mg/kg	P20	0.895	0.051	0.208	1.707	2.864	0.040	6.463	0.007

	P21	0.826	ND	0.206	1.576	2.860	0.046	5.291	0.007
	P26	0.825	0.025	0.158	1.383	3.248	0.031	4.111	0.008
	P34	0.756	0.083	0.176	1.599	3.799	0.047	3.905	0.011
	P38	0.859	0.078	0.097	1.649	3.226	0.047	5.182	0.012
	P40	0.828	0.116	0.201	1.360	2.680	0.045	5.619	0.006
	Mean	**0.802**	**0.072**	**0.159**	**1.600**	**3.033**	**0.042**	**4.811**	**0.009**
	SD	**0.051**	**0.030**	**0.039**	**0.145**	**0.299**	**0.011**	**0.806**	**0.002**
	G2	0.861	0.072	0.126	1.573	3.257	0.045	4.938	0.005
	G8	0.789	0.075	0.156	1.750	2.764	0.042	4.000	0.007
	G9	0.735	0.046	0.148	1.419	2.615	0.055	4.059	0.012
	G17	0.762	0.066	0.114	1.680	3.094	0.037	4.088	0.009
	G19	0.874	0.131	0.207	2.025	3.029	0.040	3.778	0.009
100 mg/kg	G20	0.692	0.121	0.129	1.456	2.677	0.048	4.068	0.010
	G24	0.787	0.039	0.151	1.398	2.910	0.046	4.214	0.017
	G27	0.862	0.063	0.231	1.790	3.090	0.048	4.595	0.022
	G28	0.697	0.089	0.180	1.616	3.225	0.077	4.338	0.016
	G32	0.799	0.114	0.176	1.653	2.948	0.091	4.666	0.012
	G39	0.801	0.060	0.156	1.675	2.922	0.060	5.513	0.013
	Mean	**0.787**	**0.080**	**0.161**	**1.640**	**2.957**	**0.054**	**4.387**	**0.012**
	SD	**0.063**	**0.031**	**0.035**	**0.182**	**0.210**	**0.017**	**0.504**	**0.005**
500 mg/kg	B18	0.552	0.045	0.072	1.411	2.866	0.019	2.218	0.010

ND = no data

Table L-3
10 Week Individual Organ Mass
F1 Generation Female Quail

ABSOLUTE ORGAN MASS (GRAMS)

Group	Animal ID	Brain	Bursa	Heart	Liver	Ovaries	Oviduct	Spleen	Thyroid
	Y52	0.849	0.024	1.488	5.840	5.387	6.324	0.125	0.019
	Y57	0.774	0.047	1.064	5.079	4.766	5.704	0.041	0.010
	Y64	0.742	0.040	1.562	3.377	0.895	1.949	0.040	0.010
	Y65	0.860	0.038	1.268	5.399	5.988	6.847	0.044	0.009
0 mg/kg	Y67	0.772	0.034	1.297	5.841	6.734	7.155	0.104	0.007
	Y70	0.804	0.050	1.678	6.222	5.433	5.229	0.025	0.012
	Y73	0.814	0.017	1.214	7.928	4.183	5.715	0.060	0.014
	Y78	0.769	0.044	1.176	5.274	1.900	5.132	0.038	0.001
	Y80	0.803	0.047	0.876	5.663	3.363	6.126	0.068	0.015
	Y82	0.922	0.019	1.515	5.495	9.143	8.025	0.048	0.020
	Y83	0.834	0.055	0.919	7.206	3.020	5.267	0.055	0.012
	Y85	0.812	0.072	1.517	4.864	5.352	7.991	0.062	0.004
	Y86	0.809	0.042	1.136	6.752	6.173	6.892	0.051	0.024
	Mean	**0.813**	**0.041**	**1.285**	**5.765**	**4.795**	**6.027**	**0.059**	**0.012**
	SD	**0.047**	**0.015**	**0.253**	**1.133**	**2.165**	**1.568**	**0.028**	**0.006**
	P52	0.844	0.035	1.540	4.844	7.076	6.548	0.071	0.014
	P54	0.768	0.038	1.253	7.064	5.953	6.239	0.105	0.016
	P57	0.839	0.060	1.355	5.138	2.374	6.900	0.105	0.007
	P58	0.711	0.026	1.032	4.659	6.188	5.330	0.069	0.019
20 mg/kg	P60	0.797	0.051	1.104	5.012	5.273	5.985	0.052	0.016

	P61	0.818	0.025	1.416	6.451	6.110	5.818	0.056	0.011
	P62	0.749	0.053	1.479	5.554	3.962	5.677	0.035	0.014
	P65	0.775	0.026	1.168	8.124	6.327	6.586	0.086	0.008
	P70	0.799	0.047	1.206	5.622	5.354	5.756	0.091	0.018
	P73	0.769	0.075	1.540	5.584	4.250	5.980	0.087	0.011
	P77	0.832	0.046	1.163	5.611	3.559	5.520	0.041	0.011
	P78	0.792	0.059	1.220	5.110	4.579	6.747	0.045	0.006
	P86	0.815	0.041	1.182	5.464	4.628	5.329	0.114	0.010
	P89	0.794	0.071	1.475	8.512	6.212	6.564	0.080	0.016
	P92	0.804	0.032	1.362	6.913	4.709	6.269	0.108	0.010
	P94	0.817	0.021	1.622	5.424	8.536	7.628	0.071	0.022
	P95	0.762	0.082	1.452	5.718	7.046	7.102	0.138	0.013
	Mean	**0.793**	**0.046**	**1.328**	**5.930**	**5.420**	**6.234**	**0.080**	**0.013**
	SD	**0.035**	**0.018**	**0.175**	**1.116**	**1.500**	**0.648**	**0.029**	**0.004**
	G53	0.831	0.038	1.366	5.275	5.807	8.678	0.056	0.011
	G54	0.729	0.021	1.585	5.234	4.831	6.398	0.047	0.008
	G59	0.774	0.062	1.231	7.031	4.667	5.820	0.083	0.010
	G63	0.787	0.048	1.199	5.828	5.922	6.631	0.092	0.023
100 mg/kg	G69	0.767	0.032	1.342	4.382	4.792	5.541	0.041	0.006
	G71	0.825	0.022	1.621	4.938	4.945	8.144	0.050	0.008
	G72	0.781	0.035	1.457	4.773	9.527	5.890	0.115	0.009
	G75	0.756	0.037	1.108	5.454	4.887	7.264	0.105	0.013
	G77	0.811	0.038	1.582	6.144	8.293	6.826	0.039	0.022
	G78	0.800	0.034	1.564	5.953	8.363	5.923	0.051	0.005
	G79	0.685	0.025	1.381	7.028	8.324	7.454	0.074	0.012
	G80	0.746	0.051	1.482	5.403	5.734	5.893	0.071	0.015
	G82	0.833	0.051	1.350	7.101	7.627	6.457	0.118	0.008

G84	0.857	0.021	1.210	6.491	6.025	5.961	0.054	0.007
G88	0.927	0.061	1.220	6.110	8.668	6.701	0.141	0.011
Mean	**0.794**	**0.038**	**1.380**	**5.810**	**6.561**	**6.639**	**0.076**	**0.011**
SD	**0.058**	**0.014**	**0.164**	**0.850**	**1.707**	**0.909**	**0.032**	**0.005**

Table L-4
10 Week Individual Organ Mass
F1 Generation Male Quail

ABSOLUTE ORGAN MASS (GRAMS)

Group	Animal ID	Brain	Bursa	Epididymis	Heart	Liver	Spleen	Testes	Thyroid
	Y51	0.788	0.168	0.067	1.505	2.904	0.054	3.544	0.013
	Y53	0.856	0.071	0.137	1.950	2.599	0.053	4.771	0.009
	Y54	0.701	0.094	0.113	1.199	2.588	0.024	3.237	0.008
	Y58	0.814	0.114	0.090	1.476	2.572	0.015	4.045	0.013
0 mg/kg	Y60	0.716	0.055	0.144	1.359	2.576	0.046	4.341	0.012
	Y63	0.821	0.061	0.165	1.312	2.426	0.041	4.687	0.013
	Y66	0.816	0.064	0.133	1.400	2.221	0.045	4.006	0.011
	Y69	0.793	0.071	0.098	1.478	2.393	0.040	3.889	0.013
	Y71	0.798	0.079	0.083	1.369	1.857	0.022	3.705	0.011
	Y72	0.819	0.102	0.149	1.474	2.628	0.049	5.407	0.011
	Y74	0.800	0.072	0.157	1.371	2.202	0.052	4.820	0.010
	Y81	0.795	0.085	0.160	1.645	2.685	0.052	4.215	0.017
	Y84	0.776	0.036	0.161	1.676	2.537	0.052	4.265	0.007
	Y87	0.833	0.100	0.151	1.267	3.128	0.058	5.043	0.012
	Y88	0.779	0.037	0.166	1.214	3.143	0.058	5.257	0.003
	Mean	**0.794**	**0.081**	**0.132**	**1.446**	**2.564**	**0.044**	**4.349**	**0.011**
	SD	**0.041**	**0.033**	**0.033**	**0.196**	**0.337**	**0.013**	**0.637**	**0.003**
	P51	0.774	0.170	0.179	1.328	3.302	0.044	3.996	0.013
	P59	0.810	0.102	0.124	1.401	2.768	0.049	5.270	0.011

	P63	0.789	0.245	0.131	1.638	3.050	0.057	5.200	0.008
	P67	0.803	0.103	0.127	1.700	2.873	0.045	4.046	0.011
	P68	0.686	0.054	0.097	1.415	2.349	0.042	4.165	0.008
	P69	0.809	0.115	0.113	1.493	2.430	0.075	4.689	0.011
20 mg/kg	P72	0.856	0.101	0.147	1.340	2.948	0.054	4.695	0.006
	P74	0.791	0.083	0.163	1.412	2.916	0.042	4.751	0.009
	P75	0.838	0.117	0.096	1.534	2.354	0.033	3.428	0.008
	P79	0.807	0.142	0.148	1.360	3.245	0.043	3.345	0.008
	P80	0.797	0.085	0.135	1.550	2.790	0.045	4.233	0.009
	P81	0.830	0.132	0.131	1.388	2.533	0.054	3.380	0.014
	P84	0.795	0.075	0.125	1.702	3.607	0.088	5.369	0.009
	P90	0.786	0.057	0.113	1.639	2.722	0.066	5.031	0.010
	P91	0.819	0.028	0.126	1.540	2.399	0.028	4.674	0.008
	P96	0.943	0.097	0.151	1.334	2.782	0.042	5.526	0.011
	P98	0.778	0.083	0.141	1.594	2.709	0.034	4.722	0.021
	P99	0.688	0.100	0.113	1.577	2.130	0.022	2.885	0.007
	Mean	**0.800**	**0.105**	**0.131**	**1.497**	**2.773**	**0.048**	**4.411**	**0.010**
	SD	**0.056**	**0.048**	**0.022**	**0.128**	**0.378**	**0.016**	**0.776**	**0.003**
	G52	0.706	0.121	0.101	1.303	2.159	0.062	3.367	0.008
	G55	0.835	0.065	0.105	1.455	3.125	0.064	4.805	0.004
	G57	0.758	0.073	0.101	1.288	2.166	0.085	3.446	0.011
	G58	0.849	0.045	0.150	1.545	3.197	0.036	4.895	0.006
	G62	0.905	0.092	0.125	1.316	2.033	0.037	4.007	0.003
100 mg/kg	G64	0.844	0.055	0.101	1.682	3.088	0.088	3.613	0.007
	G65	0.836	0.096	0.105	1.417	3.308	0.029	3.914	0.005
	G66	0.710	0.049	0.064	1.359	2.365	0.032	3.042	0.008
	G68	0.703	0.114	0.112	1.420	1.943	0.065	5.088	0.009

G74	0.793	0.049	0.115	1.384	2.232	0.037	3.705	0.007
G76	0.794	0.104	0.118	1.394	3.302	0.055	3.966	0.009
G81	0.758	0.009	0.121	1.429	2.955	0.083	3.867	0.009
G85	0.798	0.052	0.137	1.322	3.298	0.075	4.286	0.012
G86	0.751	0.054	0.151	1.575	2.527	0.032	4.537	0.009
G87	0.801	0.090	0.176	1.512	2.605	0.058	5.495	0.011
G90	0.836	0.055	0.157	1.441	2.473	0.034	5.329	0.012
Mean	**0.792**	**0.070**	**0.121**	**1.428**	**2.674**	**0.055**	**4.210**	**0.008**
SD	**0.058**	**0.030**	**0.028**	**0.108**	**0.499**	**0.021**	**0.739**	**0.003**

Table L-5
12 Week Individual Organ Mass/Body Mass
F0 Generation Female Quail

ORGAN MASS/BODY MASS

Group	Animal ID	Brain	Bursa	Heart	Liver	Ovaries	Oviduct	Spleen	Thyroid
	Y1	0.00484	0.00033	0.00763	0.02758	0.03480	0.03718	0.00037	0.00009
	Y4	0.00365	0.00011	0.00836	0.02572	0.04109	0.03772	0.00028	0.00009
	Y11	0.00392	0.00033	0.00888	0.03032	0.02420	0.03445	0.00030	0.00003
	Y22	0.00428	0.00011	0.00776	0.02576	0.03167	0.03193	0.00033	0.00009
0 mg/kg	Y23	0.00417	0.00009	0.00758	0.03986	0.02760	0.02702	0.00044	0.00006
	Y24	0.00483	0.00050	0.00897	0.02587	0.02794	0.03505	0.00017	0.00009
	Y29	0.00411	ND	0.00711	0.02825	0.02031	0.04274	0.00045	0.00005
	Y30	0.00472	0.00023	0.00633	0.02507	0.04314	0.03647	0.00027	0.00005
	Y34	0.00462	0.00012	0.00905	0.02778	0.02693	0.03595	0.00036	0.00008
	Y46	0.00448	0.00009	0.00666	0.02956	0.03512	0.03483	0.00028	0.00006
	Y52	0.00459	0.00015	0.00644	0.03148	0.02971	0.03709	0.00028	0.00007
	Mean	**0.00438**	**0.00021**	**0.00771**	**0.02884**	**0.03114**	**0.03549**	**0.00032**	**0.00007**
	SD	**0.00039**	**0.00014**	**0.00101**	**0.00420**	**0.00693**	**0.00387**	**0.00008**	**0.00002**
	P1	0.00403	0.00020	0.00961	0.02331	0.03059	0.02868	0.00058	0.00005
	P9	0.00422	0.00019	0.00901	0.02775	0.02660	0.03445	0.00026	0.00012
	P10	0.00423	0.00020	0.00864	0.03116	0.04377	0.04531	0.00036	0.00007
	P16	0.00359	0.00021	0.00720	0.02418	0.02817	0.03150	0.00030	0.00003
20 mg/kg	P17	0.00420	0.00050	0.01009	0.03129	0.02704	0.03701	0.00040	0.00007
	P22	0.00387	0.00049	0.00714	0.02932	0.02873	0.03326	0.00019	0.00007
	P24	0.00438	0.00019	0.00752	0.02780	0.02722	0.03559	0.00029	0.00008
	P31	0.00409	0.00029	0.00767	0.02555	0.03760	0.03720	0.00057	0.00006
	P39	0.00440	0.00012	0.00909	0.03406	0.03233	0.04020	0.00035	0.00006
	P42	0.00410	0.00064	0.00798	0.02984	0.03109	0.04024	0.00037	0.00006
	Mean	**0.00411**	**0.00030**	**0.00840**	**0.02843**	**0.03131**	**0.03634**	**0.00037**	**0.00007**

	SD	**0.00024**	**0.00018**	**0.00104**	**0.00339**	**0.00548**	**0.00480**	**0.00012**	**0.00002**
	G7	0.00454	0.00032	0.00784	0.02746	0.03732	0.03777	0.00035	0.00012
	G11	0.00366	0.00007	0.00855	0.03249	0.04148	0.03052	0.00020	0.00009
	G14	0.00451	0.00019	0.00728	0.03503	0.03428	0.03789	0.00057	0.00007
	G15	0.00424	0.00044	0.00756	0.02662	0.03577	0.03333	0.00018	0.00014
100 mg/kg	G18	0.00376	0.00025	0.00744	0.02327	0.02484	0.03407	0.00014	0.00003
	G26	0.00353	0.00025	0.00767	0.03005	0.03962	0.03580	0.00037	0.00010
	G30	0.00422	0.00019	0.00879	0.02655	0.02452	0.03371	ND	0.00008
	G31	0.00362	0.00018	0.00685	0.02470	0.02966	0.03331	0.00033	0.00003
	G35	0.00441	0.00018	0.00665	0.02960	0.03153	0.03820	0.00034	0.00009
	G38	0.00432	0.00037	0.00839	0.03187	0.02516	0.03052	0.00049	0.00008
	G40	0.00440	0.00041	0.01008	0.02832	0.03059	0.03812	0.00050	0.00005
	G42	0.00347	0.00017	0.01063	0.02817	0.03482	0.03104	0.00037	0.00007
	Mean	**0.00406**	**0.00025**	**0.00814**	**0.02868**	**0.03247**	**0.03452**	**0.00035**	**0.00008**
	SD	**0.00041**	**0.00011**	**0.00122**	**0.00335**	**0.00574**	**0.00298**	**0.00014**	**0.00003**

ND = no data

Table L-6
12 Week Individual Organ Mass/Body Mass
F0 Generation Male Quail

ORGAN MASS/BODY MASS

Group	Animal ID	Brain	Bursa	Epididymis	Heart	Liver	Spleen	Testes	Thyroid
	Y2	0.00477	0.00074	0.00073	0.00966	0.02033	0.00021	0.02661	0.00005
	Y7	0.00529	0.00043	0.00104	0.00936	0.01671	0.00019	0.03464	0.00007
	Y8	0.00488	0.00064	0.00090	0.00884	0.01875	0.00024	0.02951	0.00007
	Y10	0.00504	0.00032	0.00081	0.01033	0.01801	0.00036	0.03011	0.00005
0 mg/kg	Y19	0.00485	0.00026	0.00112	0.00947	0.01839	0.00028	0.02570	0.00008
	Y26	0.00491	0.00054	0.00139	0.00948	0.01758	0.00037	0.03603	0.00015
	Y35	0.00460	0.00039	0.00099	0.00936	0.01566	0.00018	0.03403	0.00002
	Y38	0.00470	0.00039	0.00095	0.01074	0.02173	0.00018	0.03631	0.00005
	Y39	0.00513	0.00054	0.00081	0.00964	0.01600	0.00028	0.02602	0.00001
	Y42	0.00471	0.00023	0.00084	0.00862	0.01525	0.00022	0.02518	0.00005
	Y51	0.00489	0.00070	0.00093	0.01058	0.01503	0.00050	0.03092	0.00011
	Mean	**0.00489**	**0.00047**	**0.00095**	**0.00965**	**0.01759**	**0.00027**	**0.03046**	**0.00006**
	SD	**0.00020**	**0.00017**	**0.00019**	**0.00067**	**0.00214**	**0.00010**	**0.00426**	**0.00004**
	P3	0.00432	0.00042	0.00114	0.00945	0.01624	0.00021	0.02829	0.00003
	P5	0.00458	0.00021	0.00077	0.00909	0.01811	0.00019	0.02911	0.00005
	P12	0.00476	0.00054	0.00070	0.01154	0.01750	0.00041	0.02738	0.00005
	P14	0.00542	0.00063	0.00100	0.01004	0.01866	0.00017	0.02671	0.00005
	P18	0.00396	0.00061	0.00080	0.00984	0.01691	0.00025	0.02092	0.00006
20 mg/kg	P19	0.00502	0.00026	0.00079	0.00991	0.01952	0.00029	0.03151	0.00009
	P20	0.00486	0.00028	0.00113	0.00926	0.01554	0.00022	0.03507	0.00004
	P21	0.00522	ND	0.00130	0.00996	0.01808	0.00029	0.03345	0.00004
	P26	0.00482	0.00015	0.00092	0.00808	0.01897	0.00018	0.02401	0.00005
	P34	0.00414	0.00045	0.00096	0.00876	0.02082	0.00026	0.02140	0.00006
	P38	0.00526	0.00048	0.00059	0.01010	0.01977	0.00029	0.03175	0.00007

	P40	0.00486	0.00068	0.00118	0.00798	0.01572	0.00026	0.03296	0.00004
	Mean	**0.00477**	**0.00043**	**0.00094**	**0.00950**	**0.01799**	**0.00025**	**0.02855**	**0.00005**
	SD	**0.00045**	**0.00018**	**0.00022**	**0.00097**	**0.00167**	**0.00007**	**0.00467**	**0.00002**
	G2	0.00523	0.00044	0.00077	0.00955	0.01978	0.00027	0.02998	0.00003
	G8	0.00457	0.00043	0.00090	0.01014	0.01601	0.00024	0.02317	0.00004
	G9	0.00512	0.00032	0.00103	0.00989	0.01822	0.00038	0.02829	0.00008
	G17	0.00445	0.00039	0.00067	0.00982	0.01808	0.00022	0.02389	0.00005
	G19	0.00495	0.00074	0.00117	0.01147	0.01715	0.00023	0.02139	0.00005
100 mg/kg	G20	0.00420	0.00073	0.00078	0.00883	0.01623	0.00029	0.02467	0.00006
	G24	0.00552	0.00027	0.00106	0.00980	0.02039	0.00032	0.02953	0.00012
	G27	0.00561	0.00041	0.00150	0.01165	0.02010	0.00031	0.02990	0.00014
	G28	0.00389	0.00050	0.00100	0.00902	0.01800	0.00043	0.02421	0.00009
	G32	0.00514	0.00073	0.00113	0.01064	0.01897	0.00059	0.03003	0.00008
	G39	0.00471	0.00035	0.00092	0.00986	0.01720	0.00035	0.03245	0.00008
	Mean	**0.00485**	**0.00048**	**0.00099**	**0.01006**	**0.01819**	**0.00033**	**0.02705**	**0.00007**
	SD	**0.00054**	**0.00017**	**0.00023**	**0.00089**	**0.00149**	**0.00011**	**0.00365**	**0.00003**
500 mg/kg	B18	0.00368	0.00030	0.00048	0.00941	0.01911	0.00013	0.01479	0.00007

ND = no data

Table L-7
10 Week Individual Organ Mass/Body Mass
F1 Generation Female Quail

ORGAN MASS/BODY MASS

Group	Animal ID	Brain	Bursa	Heart	Liver	Ovaries	Oviduct	Spleen	Thyroid
	Y52	0.00446	0.00013	0.00782	0.03067	0.02829	0.03321	0.00066	0.00010
	Y57	0.00533	0.00032	0.00733	0.03500	0.03285	0.03931	0.00028	0.00007
	Y64	0.00385	0.00021	0.00811	0.01754	0.00465	0.01012	0.00021	0.00005
	Y65	0.00475	0.00021	0.00701	0.02985	0.03310	0.03785	0.00024	0.00005
0 mg/kg	Y67	0.00434	0.00019	0.00729	0.03283	0.03785	0.04022	0.00058	0.00004
	Y70	0.00418	0.00026	0.00873	0.03236	0.02825	0.02719	0.00013	0.00006
	Y73	0.00435	0.00009	0.00649	0.04235	0.02235	0.03053	0.00032	0.00007
	Y78	0.00476	0.00027	0.00728	0.03266	0.01176	0.03178	0.00024	0.00001
	Y80	0.00526	0.00031	0.00574	0.03711	0.02204	0.04014	0.00045	0.00010
	Y82	0.00464	0.00010	0.00763	0.02768	0.04606	0.04043	0.00024	0.00010
	Y83	0.00479	0.00032	0.00528	0.04141	0.01736	0.03027	0.00032	0.00007
	Y85	0.00434	0.00038	0.00810	0.02598	0.02859	0.04269	0.00033	0.00002
	Y86	0.00474	0.00025	0.00665	0.03955	0.03616	0.04037	0.00030	0.00014
	Mean	**0.00460**	**0.00023**	**0.00719**	**0.03269**	**0.02687**	**0.03416**	**0.00033**	**0.00007**
	SD	**0.00041**	**0.00009**	**0.00097**	**0.00679**	**0.01123**	**0.00878**	**0.00015**	**0.00004**
	P52	0.00460	0.00019	0.00840	0.02643	0.03860	0.03572	0.00039	0.00008
	P54	0.00393	0.00019	0.00641	0.03613	0.03045	0.03191	0.00054	0.00008
	P57	0.00485	0.00035	0.00783	0.02970	0.01372	0.03988	0.00061	0.00004
	P58	0.00450	0.00016	0.00653	0.02949	0.03916	0.03373	0.00044	0.00012
20 mg/kg	P60	0.00467	0.00030	0.00647	0.02936	0.03089	0.03506	0.00030	0.00009
	P61	0.00420	0.00013	0.00728	0.03315	0.03140	0.02990	0.00029	0.00006
	P62	0.00383	0.00027	0.00755	0.02837	0.02023	0.02899	0.00018	0.00007
	P65	0.00414	0.00014	0.00624	0.04342	0.03382	0.03520	0.00046	0.00004
	P70	0.00444	0.00026	0.00670	0.03125	0.02976	0.03200	0.00051	0.00010

	P73	0.00441	0.00043	0.00883	0.03200	0.02436	0.03427	0.00050	0.00006
	P77	0.00488	0.00027	0.00682	0.03289	0.02086	0.03236	0.00024	0.00006
	P78	0.00447	0.00033	0.00688	0.02884	0.02584	0.03808	0.00025	0.00003
	P86	0.00426	0.00021	0.00618	0.02856	0.02419	0.02786	0.00060	0.00005
	P89	0.00370	0.00033	0.00687	0.03965	0.02893	0.03057	0.00037	0.00007
	P92	0.00452	0.00018	0.00766	0.03886	0.02647	0.03524	0.00061	0.00006
	P94	0.00363	0.00009	0.00720	0.02407	0.03789	0.03386	0.00032	0.00010
	P95	0.00388	0.00042	0.00740	0.02913	0.03589	0.03618	0.00070	0.00007
	Mean	**0.00429**	**0.00025**	**0.00713**	**0.03184**	**0.02897**	**0.03358**	**0.00043**	**0.00007**
	SD	**0.00039**	**0.00010**	**0.00075**	**0.00507**	**0.00705**	**0.00319**	**0.00015**	**0.00002**
100 mg/kg	G53	0.00501	0.00023	0.00824	0.03182	0.03502	0.05234	0.00034	0.00007
	G54	0.00419	0.00012	0.00910	0.03006	0.02775	0.03675	0.00027	0.00005
	G59	0.00426	0.00034	0.00678	0.03872	0.02570	0.03205	0.00046	0.00006
	G63	0.00431	0.00026	0.00656	0.03188	0.03240	0.03627	0.00050	0.00013
	G69	0.00413	0.00017	0.00723	0.02361	0.02582	0.02985	0.00022	0.00003
	G71	0.00433	0.00012	0.00851	0.02593	0.02597	0.04277	0.00026	0.00004
	G72	0.00426	0.00019	0.00794	0.02601	0.05192	0.03210	0.00063	0.00005
	G75	0.00408	0.00020	0.00598	0.02943	0.02637	0.03920	0.00057	0.00007
	G77	0.00429	0.00020	0.00836	0.03247	0.04383	0.03608	0.00021	0.00012
	G78	0.00421	0.00018	0.00824	0.03136	0.04406	0.03121	0.00027	0.00003
	G79	0.00363	0.00013	0.00732	0.03724	0.04411	0.03950	0.00039	0.00006
	G80	0.00387	0.00026	0.00768	0.02801	0.02973	0.03055	0.00037	0.00008
	G82	0.00423	0.00026	0.00685	0.03603	0.03870	0.03276	0.00060	0.00004
	G84	0.00475	0.00012	0.00670	0.03594	0.03336	0.03301	0.00030	0.00004
	G88	0.00471	0.00031	0.00619	0.03102	0.04400	0.03402	0.00072	0.00006
	Mean	**0.00428**	**0.00021**	**0.00745**	**0.03130**	**0.03525**	**0.03590**	**0.00041**	**0.00006**
	SD	**0.00034**	**0.00007**	**0.00093**	**0.00438**	**0.00862**	**0.00586**	**0.00016**	**0.00003**

Table L-8
10 Week Individual Organ Mass/Body Mass
F1 Generation Male Quail

ORGAN MASS/BODY MASS

Group	Animal ID	Brain	Bursa	Epididymis	Heart	Liver	Spleen	Testes	Thyroid
	Y51	0.00520	0.00111	0.00066	0.00993	0.01917	0.00036	0.02339	0.00009
	Y53	0.00532	0.00044	0.00113	0.01213	0.01616	0.00033	0.02967	0.00006
	Y54	0.00480	0.00064	0.00086	0.00821	0.01771	0.00016	0.02216	0.00005
	Y58	0.00568	0.00080	0.00087	0.01030	0.01795	0.00010	0.02823	0.00009
0 mg/kg	Y60	0.00492	0.00038	0.00105	0.00934	0.01770	0.00032	0.02984	0.00008
	Y63	0.00545	0.00040	0.00122	0.00871	0.01610	0.00027	0.03110	0.00009
	Y66	0.00547	0.00043	0.00107	0.00938	0.01489	0.00030	0.02685	0.00007
	Y69	0.00527	0.00047	0.00069	0.00981	0.01589	0.00027	0.02582	0.00009
	Y71	0.00604	0.00060	0.00087	0.01036	0.01406	0.00017	0.02805	0.00008
	Y72	0.00539	0.00067	0.00111	0.00970	0.01730	0.00032	0.03560	0.00007
	Y74	0.00546	0.00049	0.00111	0.00936	0.01504	0.00036	0.03292	0.00007
	Y81	0.00452	0.00048	0.00081	0.00935	0.01526	0.00030	0.02395	0.00010
	Y84	0.00500	0.00023	0.00095	0.01079	0.01634	0.00033	0.02746	0.00005
	Y87	0.00556	0.00067	0.00088	0.00845	0.02087	0.00039	0.03364	0.00008
	Y88	0.00496	0.00024	0.00115	0.00772	0.01999	0.00037	0.03344	0.00002
	Mean	**0.00527**	**0.00054**	**0.00096**	**0.00957**	**0.01696**	**0.00029**	**0.02881**	**0.00007**
	SD	**0.00038**	**0.00022**	**0.00017**	**0.00110**	**0.00196**	**0.00008**	**0.00403**	**0.00002**
	P51	0.00446	0.00098	0.00109	0.00766	0.01904	0.00025	0.02304	0.00007
	P59	0.00536	0.00068	0.00090	0.00928	0.01833	0.00032	0.03490	0.00007
	P63	0.00478	0.00148	0.00089	0.00993	0.01848	0.00035	0.03152	0.00005
	P67	0.00449	0.00058	0.00066	0.00951	0.01607	0.00025	0.02263	0.00006
	P68	0.00458	0.00036	0.00095	0.00945	0.01568	0.00028	0.02780	0.00005
20 mg/kg	P69	0.00558	0.00079	0.00130	0.01029	0.01675	0.00052	0.03232	0.00008
	P72	0.00525	0.00062	0.00108	0.00822	0.01807	0.00033	0.02879	0.00004

	P74	0.00478	0.00050	0.00094	0.00853	0.01762	0.00025	0.02871	0.00005
	P75	0.00524	0.00073	0.00052	0.00960	0.01473	0.00021	0.02145	0.00005
	P79	0.00496	0.00087	0.00080	0.00836	0.01996	0.00026	0.02057	0.00005
	P80	0.00495	0.00053	0.00085	0.00962	0.01732	0.00028	0.02628	0.00006
	P81	0.00489	0.00078	0.00079	0.00817	0.01491	0.00032	0.01989	0.00008
	P84	0.00500	0.00047	0.00069	0.01070	0.02269	0.00055	0.03377	0.00006
	P90	0.00442	0.00032	0.00070	0.00922	0.01531	0.00037	0.02830	0.00006
	P91	0.00590	0.00020	0.00107	0.01110	0.01728	0.00020	0.03367	0.00006
	P96	0.00569	0.00059	0.00092	0.00805	0.01678	0.00025	0.03333	0.00007
	P98	0.00464	0.00049	0.00075	0.00951	0.01615	0.00020	0.02816	0.00013
	P99	0.00460	0.00067	0.00104	0.01055	0.01425	0.00015	0.01930	0.00005
	Mean	**0.00498**	**0.00065**	**0.00089**	**0.00932**	**0.01719**	**0.00030**	**0.02747**	**0.00006**
	SD	**0.00044**	**0.00029**	**0.00019**	**0.00099**	**0.00208**	**0.00010**	**0.00523**	**0.00002**
	G52	0.00443	0.00076	0.00070	0.00818	0.01355	0.00039	0.02114	0.00005
	G55	0.00516	0.00040	0.00083	0.00899	0.01930	0.00040	0.02968	0.00002
	G57	0.00542	0.00052	0.00114	0.00921	0.01548	0.00061	0.02463	0.00008
	G58	0.00514	0.00027	0.00088	0.00936	0.01936	0.00022	0.02965	0.00004
	G62	0.00627	0.00064	0.00105	0.00911	0.01408	0.00026	0.02775	0.00002
100 mg/kg	G64	0.00552	0.00036	0.00067	0.01101	0.02021	0.00058	0.02365	0.00005
	G65	0.00529	0.00061	0.00070	0.00897	0.02095	0.00018	0.02479	0.00003
	G66	0.00501	0.00035	0.00073	0.00958	0.01668	0.00023	0.02145	0.00006
	G68	0.00445	0.00072	0.00051	0.00898	0.01229	0.00041	0.03218	0.00006
	G74	0.00543	0.00034	0.00097	0.00947	0.01528	0.00025	0.02536	0.00005
	G76	0.00473	0.00062	0.00082	0.00830	0.01965	0.00033	0.02361	0.00005
	G81	0.00476	0.00006	0.00073	0.00898	0.01856	0.00052	0.02429	0.00006
	G85	0.00460	0.00030	0.00074	0.00763	0.01903	0.00043	0.02473	0.00007
	G86	0.00505	0.00036	0.00100	0.01059	0.01699	0.00022	0.03051	0.00006
	G87	0.00590	0.00066	0.00108	0.01114	0.01920	0.00043	0.04049	0.00008
	G90	0.00594	0.00039	0.00113	0.01024	0.01758	0.00024	0.03787	0.00009
	Mean	0.00519	0.00046	0.00085	0.00936	0.01739	0.00036	0.02761	0.00005
	SD	0.00054	0.00019	0.00019	0.00098	0.00260	0.00014	0.00555	0.00002

Table L-9
12 Week Individual Organ Mass/Brain Mass
F0 Generation Female Quail

ORGAN MASS/BRAIN MASS

Group	Animal ID	Bursa	Heart	Liver	Ovaries	Oviduct	Spleen	Thyroid
	Y1	0.067	1.577	5.702	7.196	7.686	0.076	0.019
	Y4	0.029	2.291	7.050	11.264	10.340	0.076	0.024
	Y11	0.085	2.267	7.739	6.179	8.795	0.076	0.008
	Y22	0.027	1.812	6.013	7.391	7.451	0.077	0.020
0 mg/kg	Y23	0.022	1.820	9.566	6.623	6.484	0.105	0.015
	Y24	0.104	1.858	5.360	5.789	7.262	0.035	0.019
	Y29	ND	1.730	6.872	4.941	10.396	0.110	0.013
	Y30	0.048	1.340	5.308	9.135	7.723	0.058	0.012
	Y34	0.026	1.959	6.013	5.830	7.782	0.077	0.016
	Y46	0.021	1.489	6.606	7.849	7.784	0.062	0.014
	Y52	0.033	1.405	6.862	6.475	8.083	0.061	0.015
	Mean	**0.046**	**1.777**	**6.645**	**7.152**	**8.162**	**0.074**	**0.016**
	SD	**0.030**	**0.317**	**1.230**	**1.777**	**1.224**	**0.021**	**0.004**
	P1	0.049	2.386	5.790	7.598	7.123	0.144	0.014
	P9	0.046	2.137	6.582	6.310	8.169	0.062	0.028
	P10	0.048	2.044	7.372	10.354	10.718	0.085	0.016
	P16	0.059	2.003	6.725	7.837	8.764	0.084	0.010
20 mg/kg	P17	0.120	2.406	7.459	6.445	8.822	0.096	0.018
	P22	0.127	1.843	7.570	7.419	8.587	0.050	0.017
	P24	0.044	1.719	6.349	6.216	8.127	0.067	0.019
	P31	0.070	1.876	6.248	9.195	9.097	0.139	0.015
	P39	0.028	2.067	7.743	7.350	9.139	0.080	0.013
	P42	0.157	1.946	7.274	7.578	9.810	0.089	0.015
	Mean	**0.075**	**2.043**	**6.911**	**7.630**	**8.836**	**0.090**	**0.016**

	SD	**0.044**	**0.222**	**0.660**	**1.299**	**0.977**	**0.031**	**0.005**
	G7	0.070	1.728	6.054	8.228	8.328	0.078	0.026
	G11	0.020	2.332	8.866	11.320	8.330	0.055	0.024
	G14	0.043	1.614	7.770	7.603	8.405	0.127	0.015
	G15	0.104	1.782	6.276	8.435	7.858	0.044	0.033
	G18	0.068	1.979	6.186	6.604	9.057	0.039	0.008
100 mg/kg	G26	0.071	2.169	8.502	11.211	10.131	0.104	0.028
	G30	0.045	2.085	6.298	5.815	7.995	ND	0.019
	G31	0.051	1.893	6.829	8.199	9.208	0.092	0.010
	G35	0.041	1.507	6.705	7.142	8.654	0.077	0.021
	G38	0.085	1.944	7.386	5.831	7.073	0.113	0.019
	G40	0.094	2.291	6.436	6.952	8.664	0.113	0.011
	G42	0.048	3.059	8.110	10.022	8.935	0.106	0.021
	Mean	**0.062**	**2.032**	**7.118**	**8.113**	**8.553**	**0.086**	**0.020**
	SD	**0.025**	**0.412**	**0.979**	**1.882**	**0.766**	**0.030**	**0.008**

ND = no data

Table L-10
12 Week Individual Organ Mass/Brain Mass
F0 Generation Male Quail

ORGAN MASS/BRAIN MASS

Group	Animal ID	Bursa	Epididymis	Heart	Liver	Spleen	Testes	Thyroid
	Y2	0.155	0.152	2.027	4.264	0.043	5.581	0.011
	Y7	0.081	0.197	1.769	3.158	0.035	6.546	0.013
	Y8	0.130	0.184	1.810	3.840	0.049	6.044	0.015
	Y10	0.064	0.160	2.051	3.574	0.072	5.976	0.010
0 mg/kg	Y19	0.054	0.231	1.953	3.791	0.057	5.298	0.016
	Y26	0.110	0.284	1.932	3.582	0.076	7.343	0.030
	Y35	0.084	0.214	2.033	3.401	0.039	7.391	0.004
	Y38	0.083	0.202	2.286	4.625	0.038	7.726	0.011
	Y39	0.106	0.157	1.879	3.118	0.054	5.068	0.002
	Y42	0.050	0.178	1.832	3.241	0.047	5.349	0.010
	Y51	0.142	0.190	2.164	3.075	0.102	6.325	0.022
	Mean	**0.096**	**0.195**	**1.976**	**3.606**	**0.056**	**6.241**	**0.013**
	SD	**0.035**	**0.038**	**0.157**	**0.496**	**0.020**	**0.919**	**0.008**
	P3	0.097	0.263	2.187	3.760	0.048	6.551	0.008
	P5	0.045	0.169	1.985	3.955	0.041	6.358	0.010
	P12	0.113	0.147	2.427	3.679	0.086	5.757	0.010
	P14	0.115	0.184	1.852	3.440	0.031	4.925	0.010
	P18	0.155	0.202	2.485	4.273	0.064	5.286	0.014
20 mg/kg	P19	0.051	0.157	1.976	3.892	0.057	6.283	0.017
	P20	0.057	0.232	1.907	3.200	0.045	7.221	0.008
	P21	ND	0.249	1.908	3.462	0.056	6.406	0.008
	P26	0.030	0.192	1.676	3.937	0.038	4.983	0.010
	P34	0.110	0.233	2.115	5.025	0.062	5.165	0.015
	P38	0.091	0.113	1.920	3.756	0.055	6.033	0.014

	P40	0.140	0.243	1.643	3.237	0.054	6.786	0.007
	Mean	**0.091**	**0.199**	**2.007**	**3.801**	**0.053**	**5.980**	**0.011**
	SD	**0.041**	**0.047**	**0.260**	**0.497**	**0.014**	**0.753**	**0.003**
100 mg/kg	G2	0.084	0.146	1.827	3.783	0.052	5.735	0.006
	G8	0.095	0.198	2.218	3.503	0.053	5.070	0.009
	G9	0.063	0.201	1.931	3.558	0.075	5.522	0.016
	G17	0.087	0.150	2.205	4.060	0.049	5.365	0.012
	G19	0.150	0.237	2.317	3.466	0.046	4.323	0.010
	G20	0.175	0.186	2.104	3.868	0.069	5.879	0.014
	G24	0.050	0.192	1.776	3.698	0.058	5.355	0.022
	G27	0.073	0.268	2.077	3.585	0.056	5.331	0.026
	G28	0.128	0.258	2.319	4.627	0.110	6.224	0.023
	G32	0.143	0.220	2.069	3.690	0.114	5.840	0.015
	G39	0.075	0.195	2.091	3.648	0.075	6.883	0.016
	Mean	**0.102**	**0.198**	**2.085**	**3.771**	**0.069**	**5.593**	**0.015**
	SD	**0.041**	**0.043**	**0.181**	**0.331**	**0.024**	**0.655**	**0.006**
500 mg/kg	B18	0.082	0.130	2.556	5.192	0.034	4.018	0.018

ND = no data

Table L-11
10 Week Individual Organ Mass/Brain Mass
F1 Generation Female Quail

ORGAN MASS/BRAIN MASS

Group	Animal ID	Bursa	Heart	Liver	Ovaries	Oviduct	Spleen	Thyroid
	Y52	0.028	0.008	6.879	6.345	7.449	0.147	0.022
	Y57	0.061	0.007	6.562	6.158	7.370	0.053	0.013
	Y64	0.054	0.008	4.551	1.206	2.627	0.054	0.013
	Y65	0.044	0.007	6.278	6.963	7.962	0.051	0.010
0 mg/kg	Y67	0.044	0.007	7.566	8.723	9.268	0.135	0.009
	Y70	0.062	0.009	7.739	6.757	6.504	0.031	0.015
	Y73	0.021	0.006	9.740	5.139	7.021	0.074	0.017
	Y78	0.057	0.007	6.858	2.471	6.674	0.049	0.001
	Y80	0.059	0.006	7.052	4.188	7.629	0.085	0.019
	Y82	0.021	0.008	5.960	9.916	8.704	0.052	0.022
	Y83	0.066	0.005	8.640	3.621	6.315	0.066	0.014
	Y85	0.089	0.008	5.990	6.591	9.841	0.076	0.005
	Y86	0.052	0.007	8.346	7.630	8.519	0.063	0.030
	Mean	**0.051**	**0.007**	**7.089**	**5.824**	**7.375**	**0.072**	**0.015**
	SD	**0.019**	**0.001**	**1.342**	**2.452**	**1.784**	**0.034**	**0.008**
	P52	0.041	1.825	5.739	8.384	7.758	0.084	0.017
	P54	0.049	1.632	9.198	7.751	8.124	0.137	0.021
	P57	0.072	1.615	6.124	2.830	8.224	0.125	0.008
	P58	0.037	1.451	6.553	8.703	7.496	0.097	0.027
20 mg/kg	P60	0.064	1.385	6.289	6.616	7.509	0.065	0.020
	P61	0.031	1.731	7.886	7.469	7.112	0.068	0.013
	P62	0.071	1.975	7.415	5.290	7.579	0.047	0.019
	P65	0.034	1.507	10.483	8.164	8.498	0.111	0.010
	P70	0.059	1.509	7.036	6.701	7.204	0.114	0.023

	P73	0.098	2.003	7.261	5.527	7.776	0.113	0.014
	P77	0.055	1.398	6.744	4.278	6.635	0.049	0.013
	P78	0.074	1.540	6.452	5.782	8.519	0.057	0.008
	P86	0.050	1.450	6.704	5.679	6.539	0.140	0.012
	P89	0.089	1.858	10.720	7.824	8.267	0.101	0.020
	P92	0.040	1.694	8.598	5.857	7.797	0.134	0.012
	P94	0.026	1.985	6.639	10.448	9.337	0.087	0.027
	P95	0.108	1.906	7.504	9.247	9.320	0.181	0.017
	Mean	**0.059**	**1.674**	**7.491**	**6.856**	**7.864**	**0.101**	**0.017**
	SD	**0.024**	**0.216**	**1.462**	**1.919**	**0.795**	**0.037**	**0.006**
	G53	0.046	1.644	6.348	6.988	10.443	0.067	0.013
	G54	0.029	2.174	7.180	6.627	8.776	0.064	0.011
	G59	0.080	1.590	9.084	6.030	7.519	0.107	0.013
	G63	0.061	1.524	7.405	7.525	8.426	0.117	0.029
	G69	0.042	1.750	5.713	6.248	7.224	0.053	0.008
100 mg/kg	G71	0.027	1.965	5.985	5.994	9.872	0.061	0.010
	G72	0.045	1.866	6.111	12.198	7.542	0.147	0.012
	G75	0.049	1.466	7.214	6.464	9.608	0.139	0.017
	G77	0.047	1.951	7.576	10.226	8.417	0.048	0.027
	G78	0.043	1.955	7.441	10.454	7.404	0.064	0.006
	G79	0.036	2.016	10.260	12.152	10.882	0.108	0.018
	G80	0.068	1.987	7.243	7.686	7.899	0.095	0.020
	G82	0.061	1.621	8.525	9.156	7.752	0.142	0.010
	G84	0.025	1.412	7.574	7.030	6.956	0.063	0.008
	G88	0.066	1.316	6.591	9.351	7.229	0.152	0.012
	Mean	**0.048**	**1.749**	**7.350**	**8.275**	**8.397**	**0.095**	**0.014**
	SD	**0.016**	**0.259**	**1.215**	**2.161**	**1.257**	**0.038**	**0.007**

Table L-12
10 Week Individual Organ Mass/Brain Mass
F1 Generation Male Quail

ORGAN MASS/BRAIN MASS

Group	Animal ID	Bursa	Epididymis	Heart	Liver	Spleen	Testes	Thyroid
	Y51	0.213	0.127	1.910	3.685	0.069	4.497	0.016
	Y53	0.083	0.213	2.278	3.036	0.062	5.574	0.011
	Y54	0.134	0.178	1.710	3.692	0.034	4.618	0.011
	Y58	0.140	0.152	1.813	3.160	0.018	4.969	0.016
0 mg/kg	Y60	0.077	0.214	1.898	3.598	0.064	6.063	0.017
	Y63	0.074	0.224	1.598	2.955	0.050	5.709	0.016
	Y66	0.078	0.195	1.716	2.722	0.055	4.909	0.013
	Y69	0.090	0.131	1.864	3.018	0.050	4.904	0.016
	Y71	0.099	0.144	1.716	2.327	0.028	4.643	0.014
	Y72	0.125	0.206	1.800	3.209	0.060	6.602	0.013
	Y74	0.090	0.204	1.714	2.753	0.065	6.025	0.013
	Y81	0.107	0.180	2.069	3.377	0.065	5.302	0.021
	Y84	0.046	0.189	2.160	3.269	0.067	5.496	0.009
	Y87	0.120	0.158	1.521	3.755	0.070	6.054	0.014
	Y88	0.047	0.232	1.558	4.035	0.074	6.748	0.004
	Mean	**0.102**	**0.183**	**1.822**	**3.239**	**0.055**	**5.474**	**0.014**
	SD	**0.042**	**0.034**	**0.216**	**0.460**	**0.017**	**0.720**	**0.004**
	P51	0.220	0.244	1.716	4.266	0.057	5.163	0.017
	P59	0.126	0.168	1.730	3.417	0.060	6.506	0.014
	P63	0.311	0.186	2.076	3.866	0.072	6.591	0.010
	P67	0.128	0.147	2.117	3.578	0.056	5.039	0.014
	P68	0.079	0.208	2.063	3.424	0.061	6.071	0.012
20 mg/kg	P69	0.142	0.234	1.845	3.004	0.093	5.796	0.014
	P72	0.118	0.206	1.565	3.444	0.063	5.485	0.007

	P74	0.105	0.197	1.785	3.686	0.053	6.006	0.011
	P75	0.140	0.099	1.831	2.809	0.039	4.091	0.010
	P79	0.176	0.161	1.685	4.021	0.053	4.145	0.010
	P80	0.107	0.172	1.945	3.501	0.056	5.311	0.011
	P81	0.159	0.163	1.672	3.052	0.065	4.072	0.017
	P84	0.094	0.138	2.141	4.537	0.111	6.753	0.011
	P90	0.073	0.159	2.085	3.463	0.084	6.401	0.013
	P91	0.034	0.181	1.880	2.929	0.034	5.707	0.010
	P96	0.103	0.161	1.415	2.950	0.045	5.860	0.012
	P98	0.107	0.161	2.049	3.482	0.044	6.069	0.027
	P99	0.145	0.225	2.292	3.096	0.032	4.193	0.010
	Mean	**0.131**	**0.178**	**1.883**	**3.474**	**0.060**	**5.514**	**0.013**
	SD	**0.061**	**0.036**	**0.230**	**0.475**	**0.020**	**0.897**	**0.004**
100 mg/kg	G52	0.171	0.159	1.846	3.058	0.088	4.769	0.011
	G55	0.078	0.162	1.743	3.743	0.077	5.754	0.005
	G57	0.096	0.210	1.699	2.858	0.112	4.546	0.015
	G58	0.053	0.171	1.820	3.766	0.042	5.766	0.007
	G62	0.102	0.167	1.454	2.246	0.041	4.428	0.003
	G64	0.065	0.122	1.993	3.659	0.104	4.281	0.008
	G65	0.115	0.132	1.695	3.957	0.035	4.682	0.006
	G66	0.069	0.145	1.914	3.331	0.045	4.285	0.011
	G68	0.162	0.115	2.020	2.764	0.092	7.238	0.013
	G74	0.062	0.179	1.745	2.815	0.047	4.672	0.009
	G76	0.131	0.173	1.756	4.159	0.069	4.995	0.011
	G81	0.012	0.153	1.885	3.898	0.109	5.102	0.012
	G85	0.065	0.160	1.657	4.133	0.094	5.371	0.015
	G86	0.072	0.198	2.097	3.365	0.043	6.041	0.012
	G87	0.112	0.182	1.888	3.252	0.072	6.860	0.014
	G90	0.066	0.190	1.724	2.958	0.041	6.374	0.014
	Mean	**0.089**	**0.164**	**1.808**	**3.373**	**0.069**	**5.323**	**0.010**
	SD	**0.041**	**0.026**	**0.159**	**0.559**	**0.028**	**0.927**	**0.004**

Appendix M

Individual and Summary Sperm Data

Table M-1
12 Week Individual Sperm Concentration (M/ml)
F0 Generation Male Quail

Group	Animal ID	1	2	3	4	5	Mean
	Y2	11.0	6.8	5.8	7.8	6.8	7.6
0 mg/kg	Y7	8.4	3.7	5.8	5.8	4.7	5.7
	Y8	3.7	9.9	10.5	5.2	5.2	6.9
	Y10	4.7	8.4	7.8	7.3	6.3	6.9
	Y19	6.3	12.0	10.5	8.9	9.4	9.4
	Y26	4.2	5.2	5.2	6.8	5.2	5.3
	Y35	7.8	5.2	4.2	6.3	11.5	7.0
	Y38	9.9	8.4	15.7	14.1	9.4	11.5
	Y39	5.8	4.2	13.6	6.3	3.7	6.7
	Y42	1.0	1.6	2.1	2.6	1.0	1.7
	Y51	4.2	7.8	10.5	7.3	5.8	7.1
						Mean	**6.9**
						SD	**2.4**
	P3	8.9	14.6	11.5	11.5	16.7	12.6
20 mg/kg	P5	2.6	2.6	2.6	3.1	4.7	3.1
	P12	0.5	3.7	3.1	3.7	3.7	2.9
	P14	8.9	4.2	6.8	9.4	7.8	7.4
	P18	2.6	3.7	4.7	6.8	20.4	7.6
	P19	3.7	6.8	5.8	7.3	5.8	5.9
	P20	5.2	11.0	7.8	5.8	4.2	6.8
	P21	4.7	6.8	4.2	2.1	3.1	4.2
	P26	2.1	2.6	4.2	2.6	8.4	4.0
	P34	5.8	1.0	4.7	6.3	3.7	4.3
	P38	4.7	6.3	2.1	9.4	2.1	4.9
	P40	6.8	4.2	3.1	4.7	2.6	4.3
						Mean	**5.7**
						SD	**2.7**
	G2	2.1	3.7	2.6	5.2	3.1	3.3
100 mg/kg	G8	8.9	5.8	7.8	8.4	6.3	7.4
	G9	8.9	6.3	5.2	3.7	7.8	6.4
	G17	1.6	1.6	4.7	6.3	2.1	3.3
	G19	5.8	8.4	3.1	8.4	3.7	5.9
	G20	5.2	6.3	7.8	3.1	6.3	5.7
	G24	3.1	2.1	4.2	2.1	1.6	2.6
	G27	11.0	9.4	8.9	9.9	10.5	9.9
	G28	5.8	6.8	3.1	2.1	4.2	4.4
	G32	12.0	9.4	7.8	9.9	7.8	9.4
	G39	8.9	6.8	7.3	5.8	4.2	6.6
						Mean	**5.9**
						SD	**2.4**

500 mg/kg	B18	1.0	2.6	2.6	2.6	1.0	**2.0**

Table M-2
10 Week Individual Sperm Concentration (M/ml)
F1 Generation Male Quail

Group	Animal ID	1	2	3	4	Mean
	Y51	2.9	1.4	2.9	5.4	3.2
0 mg/kg	Y53	3.6	2.2	1.1	3.6	2.6
	Y54	3.6	3.6	2.2	4.3	3.4
	Y58	1.4	2.2	4.3	1.8	2.4
	Y60	7.9	5.4	5.1	6.1	6.1
	Y63	2.9	1.4	1.1	5.1	2.6
	Y66	4.0	2.2	5.1	2.9	3.6
	Y69	4.0	2.9	2.5	2.2	2.9
	Y71	5.1	2.9	4.0	2.2	3.6
	Y72	2.5	1.8	3.6	6.1	3.5
	Y74	4.7	4.0	2.9	3.6	3.8
	Y81	2.5	2.5	2.2	4.0	2.8
	Y84	2.5	1.8	3.2	6.1	3.4
	Y87	2.2	3.2	1.8	1.8	2.3
	Y88	2.5	2.9	2.5	6.1	3.5
					Mean	**3.3**
					SD	**0.9**
	P51	2.2	3.6	2.9	5.4	3.5
	P59	3.6	2.9	2.5	5.4	3.6
20 mg/kg	P63	2.9	2.9	3.2	4.3	3.3
	P67	1.4	1.8	2.9	5.4	2.9
	P68	3.6	4.0	2.2	3.2	3.3
	P69	9.7	3.2	5.1	2.9	5.2
	P72	4.3	1.4	1.8	4.0	2.9
	P74	3.2	3.6	4.0	6.1	4.2
	P75	1.8	2.5	2.2	4.0	2.6
	P79	2.5	2.2	1.8	4.7	2.8
	P80	2.2	1.8	3.2	6.1	3.3
	P81	2.9	1.4	2.9	4.3	2.9
	P84	2.9	4.0	3.6	4.0	3.6
	P90	2.5	3.2	2.2	1.4	2.3
	P91	6.1	2.9	7.2	4.0	5.1
	P96	1.8	3.2	2.5	4.7	3.1
	P98	3.2	2.2	2.5	4.3	3.1
	P99	4.3	2.9	2.5	2.2	3.0
					Mean	**3.4**
					SD	**0.8**
	G52	4.0	2.5	6.1	2.5	3.8
	G55	3.2	2.9	2.3	4.0	3.1
100 mg/kg	G57	7.9	6.5	4.0	3.2	5.4

G58	2.5	2.9	1.4	2.5	2.3
G62	3.2	5.4	2.5	5.1	4.1
G64	2.5	3.6	3.2	6.1	3.9
G65	3.6	5.1	2.2	6.1	4.3
G66	4.0	3.2	4.7	4.0	4.0
G68	3.2	3.2	5.1	2.2	3.4
G74	3.6	2.9	5.4	2.2	3.5
G76	3.6	3.2	2.2	3.6	3.2
G81	3.6	1.1	1.4	4.3	2.6
G85	2.2	1.8	1.1	5.1	2.6
G86	4.0	1.8	4.3	3.2	3.3
G87	9.0	1.8	8.3	5.1	6.1
G90	2.9	1.8	4.7	2.5	3.0
				Mean	**3.6**
				SD	**1.0**

Appendix N

Pathology Report A

PATHOLOGY REPORT

FOR

Study Title

One-Generation Reproductive Toxicity Test in Japanese quail (*Coturnix japonica)* using 3-nitro-1,2,4-triazol-5-one (NTO)

Protocol Number 80-14-07-02

Prepared by

Erica Eggers Carroll, DVM, PhD, Diplomate ACVP

23 September, 2016

TABLE OF CONTENTS

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This pathology investigation was conducted in a manner consistent with the principles of the United States Environmental Protection Agency (USEPA) Good Laboratory Practice regulations of the Toxic Substances Control Act (TSCA), as detailed in 40 CFR Part 792, plus amendments.

CARROLL.ERICA.E.1027432413

Digitally signed by CARROLL.ERICA.E.1027432413
DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USA, cn=CARROLL.ERICA.E.1027432413
Date: 2016.09.14 12:25:19 -04'00'

14 September 2016

Erica E. Carroll, DVM, PhD, Diplomate ACVP
LTC, VC
Study Pathologist
Toxicology Directorate
Army Public Health Center (Provisional)

QUALITY ASSURANCE STATEMENT

The following critical phases were audited by the APHC Quality Systems and Regulatory Compliance Office (QSARC), Laboratory and Toxicology Accreditation and Compliance Office (LTACO):

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Pathology Contributing Scientist Inspection -Interim Pathology Report GLP Standard Regulation Review	08/17/2016	09/14/2016
Pathology Contributing Scientist Inspection – Interim - Summary Data and Summary Table Review	08/17/2016	09/14/2016
Pathology Contributing Scientist Inspection- Second Interim Pathology Report GLP Standard Regulation Review	09/14/2016	09/14/2016
Pathology Contributing Scientist Inspection – Second Interim Summary Data and Summary Table Review	09/14/2016	09/14/2016
Pathology Contributing Scientist Inspection – Final Pathology Report GLP Standard Regulation Review	09/22/2016	09/23/2016
Pathology Contributing Scientist Inspection – Final Summary Data and Summary Table Review	09/22/2016	09/23/2016

Note 1 *All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.*

Note 2 *In addition to the study specific critical phase inspections listed here, general facility and process based inspections not specifically related to this study are done monthly or annually in accordance with QSARC, LTACO Standing Operating Procedures.*

Note 3 *This report has been audited by the Quality Assurance Unit (QSARC, LTACO) and is considered to be on accurate account of the data generated and of the procedures followed*

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2016 09 22
Date

INTRODUCTION

3-nitro-1,2,4-triazol-5-one (NTO) is being considered as a less sensitive munition for use by the military. To assess its toxicity in the environment and as a potential reproductive toxicant, NTO was administered to F0 (parental) generation Japanese quail on a daily basis beginning at two weeks of age. Based on the results of an avian acute oral toxicity test, the dose levels for the one-generation study were 1000, 500, 100, 20, and 0 mg/kg-d (control). NTO was administered in corn oil via oral gavage. F1 birds were exposed to NTO *in ovo* via maternal deposition. Oral treatment of the F1 generation began at day 2 of age and continued until sacrifice at 70 days of age. Gross and histologic examination of select tissues was performed.

METHODS

All tissues, with the exception of testis and epididymis, were fixed in formalin, trimmed into cassettes, processed automatically, embedded in paraffin, sectioned via microtome to a thickness of 4-5 um, and stained with hematoxylin and eosin using routine an automatic stainer. Testis and epididymis were fixed in modified Davidson's fixative, which was replaced with ethanol after 24 hours, processed, paraffin-embedded, and sectioned identically to the other tissues, but then stained with periodic acid-Schiff stain. Tissues included: brain, liver, kidney (cranial, middle, caudal divisions), heart, pancreas, spleen, bursa, thyroid gland, oviduct (infundibulum, magnum, isthmus, shell gland, vagina) testis, and epididymis.

STATISTICS

All of the 1000 mg/kg-day dose group (males and females) and all but one male of the 500 mg/kg-day dose group either died or were euthanized for humane considerations outlined in the protocol. Tissues from one relatively age-matched male F0 bird (#293, 31 days old) but no female F0 age-matched controls were present. These birds were evaluated to attempt to ascertain cause of death. Animals of the remaining treatment groups surviving to scheduled sacrifice were then evaluated. With the possible exception of the bursa in F1 males, birds exposed to 100mg/kg-d NTO did not exhibit lesions above background level, therefore statistical analyses were not performed.

RESULTS

MALES

Parental Generation (F0): All twenty F0 male birds from the 1000 mg/kg-day dose group and eighteen of nineteen 500 mg/kg-day NTO-exposed birds died or had to be euthanized prior to the end of the study, at between 31–39 days of age and 32-86 days of age, respectively (Appendix B, page 19). One bird from the 500 mg/kg-day dose group survived to scheduled sacrifice (#348, 86 days). There were no test article-related lesions in somatic tissues relative to the 31-day old or 86-day old control F0 male quails.

Tissues from animals euthanized before schedule sacrifice were not always available for histologic evaluation. Birds found dead or autolysed were not preserved. However, fifteen of eighteen testes of 1000 mg/k-day NTO-exposed quail and testes of thirteen of eighteen birds exposed to 500mg/kg-day of NTO were smaller (estimated 10 – 50 %) than the 31-day-old vehicle control bird (Figure 1A). The one 86-day-old 500 mg/kg-day bird (#348) testis was moderately smaller than the 86-day old controls (Figure 1B). Eight of eighteen 1000 mg/kg-day birds and four of eighteen 500 mg/kg-day NTO-exposed birds shed dead or multinucleate germ cells in the lumen of the testicular seminiferous tubules (Figure 2).

Testes of eleven 100 mg/kg-day NTO-exposed F0 birds, all of which survived to the end of the study (85-87 days of age), were comparable in size and morphology to the age-matched control birds.

NTO-exposed F0 bird kidneys often exhibited single-cell necrosis of epithelial cells in the distal tubules. Although most plentiful in the 1000 mg/kg-day dose group, it occurred immediately peri-mortem (Figure 3).

First Filial Generation (F1) Males: Sixteen F1 male birds exposed to 100 mg/kg-day NTO were evaluated at sacrifice at 70 days of age. Six of the birds' bursae exhibited minimal increase in tingible body macrophages or, less often, pyknotic lymphocytes or 'gap' areas suggesting a net loss of lymphocytes (involution or degeneration; Figure 4). This was observed in two of the fifteen 70-day old control male F1 quail. Testes of fourteen F1 birds were evaluable. They exhibited no reproductive tract lesions. All other examined tissues were normal.

FEMALES

Parental Generation (F0): Similar to the males, all twelve 1000 and 500 mg/kg-day female F0 birds died or had to be euthanized prior to the end of the study (Appendix B, page 23). The 1000 mg/kg-day NTO birds were 29 to 40 days old and the 500 mg/kg-day NTO birds were 33 to 56 days old. The F0 female controls were euthanized at 86 days of age; therefore age-matched controls were not available for these two groups of birds.

There were few histologic lesions in exposed birds compared to controls. One 1000 mg/kg-day female (#452) had focal cortical vacuolation of the neuropil with minimal neuronal necrosis of the (estimated) hypothalamus (Figure 5). Six 500 mg/kg-day F0 females had minimal-to-mildly congested liver.

One of ten 1000 mg/kg-day females and six of twelve 500 mg/kg-day females exhibited renal proximal tubule epithelial cell necrosis (Figure 6) but not in a dose-dependent manner and only immediately perimortem as there was no reaction of the body to this cell death. The distal renal tubules of a few exposed and control birds exhibited single cell necrosis that occurred perimortem as well.

The ovary of every 1000mg/kg-day NTO female F0 quail was mild to markedly smaller than that of control birds, but controls were 86-87 days old and the high-dose birds were less than half that age (Figure 7). Therefore it is difficult to ascertain whether the ovarian immaturity is appropriate or not for that age. The ovary of two 56-day old 500mg/kg-day NTO females was the same size as that of the older controls.

Bursas in four of nine 1000 mg/kg-d-NTO-exposed birds, five of twelve 500 mg/kg-d-NTO-exposed females and three of twelve 100 mg/kg-d-NTO exposed female F0 quail exhibited an increase in tingible body macrophages, single cell lymphocyte necrosis and paucicellular areas (Figure 4). One of ten 86-day-old control females had similar changes.

Female F0 birds exposed to 100 mg/kg-day NTO exhibited no histopathology different from that seen in controls with the exception of the liver of a single bird (#421), which had moderate portal bridging with oval cell hyperplasia.

F0 female controls and 1000 mg/kg-day NTO-exposed females exhibited minimal to marked hepatic centrilobular vacuolation on a background of fine, randomly distributed hepatocellular vacuoles. Vacuoles were not present in the 500 or 100 mg/kg-day F0 female liver sections.

F1 Generation females (F1) exposed to 100 mg/kg-day NTO had no histopathology that differed from that seen in age-matched controls with the exception of three 100 mg/kg-day NTO-exposed F1 females which had minimal to moderate hepatic portal bridging with oval cell hyperplasia compared controls. Both exposed and control birds often had minimal to marked centrilobular macrovesicular vacuolation (Figure 8).

The pancreas was rarely available for evaluation. It was not deliberately collected at necropsy but was trimmed in when available.

DISCUSSION

Birds were euthanized when they met certain predetermined criteria (e.g. loss of more than 20% body mass or an inability to stand), which were often associated with neurological signs including circling on the floor of the cage and occasionally opisthotonos. Ataxia (muscle incoordination) can result from cerebellar or brainstem pathology and opisthotonos can additionally be associated with spinal toxicity as occurs with strychnine (Watkins, 2013). No gross or clear histologic lesions were observed in the examined sections of brains or in the thoracic spinal cord. The brains of eleven of fourteen 1000 mg/kg-day male birds and twelve of fourteen 500 mg/kg-day exposed male birds exhibited vacuoles in the deep cerebellar nuclei (also known as the arbor vitae) and white matter of the same region (page 21). This finding was not present in the male longer-lived control birds and was present to a much lesser extent in females. Although the finding is reminiscent of the lesion known as avian vacuolar myelinopathy in that vacuoles are unassociated with neuronal damage or inflammation, that lesion, of unknown etiology, has only been reported in bald eagles, coots and waterfowl (Swayne et al, 2008). The finding in this study more closely resembles the common artifact comprised of irregular vacuoles in the cerebellar arbor vitae adjacent to the fourth ventricle. These postmortem changes often occur with fixatives containing alcohol (Swayne et al, 2008; Garman, 2011; Little and Rao (NTP website, undated)). Due to the high body temperature of quail, 104-106 degrees Fahrenheit during the day (Bartholomew and Dawson, 1958), autolysis begins even in moribund animals and proceeds immediately upon euthanasia. Artifactual vacuoles can be more prominent in autolysed tissues and include mild neuropil vacuolation in the gray matter. In this study the vacuoles were unaccompanied by corroborative histopathologic evidence that the observation represents a true lesion (e.g., hemorrhage, neuronopecrosis, gliosis, microgliosis, gemistocytic astrocytes). The presence of eosinophilic material in some vacuoles (Figure 9) also suggests a related common artifact known as mucocytes or Buscaino bodies (Garman, 2011). The absence of corroborative evidence of a histologic lesion leads this pathologist to interpret the vacuoles as artifact and not the source of the neurological signs. A neurological insult cannot be ruled out, however.

A possible brain ‘lesion’ was observed in F0 1000mg/k-d female bird #452. The perimortem focal cerebrocortical lesion may represent injury acquired immediately antemortem or pressure on the brain during extraction prior to fixation.

Differential diagnoses for weight loss and neurological signs in birds includes: hypoglycemia, hypocalcemia, toxicity due to zinc, salt, nitrates, nitrofurazone in some species, liver failure and others. The avian metabolism of 3-nitro-1,2,4-triazol-5-one (NTO) has not been elucidated but nitrates are known to be metabolized to nitrites which produce methemoglobinemia and neurological signs due to hypoxemia (Harrison, 1986). Mammals presenting with methemoglobinemia exhibit ataxia, anoxic seizures, brown discoloration to the blood and edema and hemorrhage in the lungs and digestive system. The lungs were not consistently examined but those inadvertently submitted attached to the cranial division of the kidney appeared mildly congested. The digestive tract was not submitted for histologic evaluation but was grossly unremarkable.

With the exception of a few pale livers, there were no gross or other histologic lesions in the examined tissues that suggested a cause of death.

It cannot be determined if daily exposures to NTO at 1000 and 500 mg/kg-day affect testicular development in juvenile Japanese quail. One relatively age-matched control (#293, euthanized at 31 days due to a reported limb injury) was available with which to compare the 1000 and 500 mg/kg-day exposure male F0 birds. This observation suggests NTO may delay development of the testes. Testes of the 31-39- day old, NTO-exposed birds were less mature than testes of the 31-day old control bird; however a single control bird may not adequately represent maturation of the population. Similarly, the 500 mg/kg-day-exposed male #348, which survived the full 86 days, had testes moderately reduced in size compared to an 86-day old control, but a single control bird makes interpretation difficult. Of note, this study examined

the effects of repeat exposure of NTO on developing, not adult birds. The main histologic feature was immaturity (characterized by seminiferous tubules without a lumen and lack of or insufficient elongating spermatids). Male Japanese quail do not produce a full complement of mature spermatozoa by 31 days of age (based on observation of the one control quail that was euthanized early and on reports in the literature (Sedqyuar, 2012)) but at least one NTO-exposed bird was producing some elongating spermatids by 35 days of age, and another was producing apparently normal sperm by 39 days old. Sedqyar et al (2012) suggest five weeks of age is precocious, therefore six weeks or later may be the average age at which male birds reach full reproductive capability. Historical data from APHC shows variation in the age at which male Japanese quail reach reproductive maturity both between and within strains. Untreated birds from a colony maintained at the University of Maryland, Animal and Avian Science Department, reached reproductive maturity at 43-44 days (unpublished data). Untreated birds from the same colony obtained two years later and used in a different study reached reproductive maturity at 45-51 days (unpublished data). However, a different strain obtained from Lake Cumberland Game Bird Farm and Hatchery used at APHC reached reproductive maturity at 34-37 days (unpublished data). It is known that quail reproductive cycle, including hormone levels and testicular development, is driven by photoperiod (Seqyar, 2012). The protocol approved 16-17 hours of light/day for chicks and adults; it is not known whether gradual changes in photoperiod is needed to facilitate reproductive maturity in this strain of *Coturnix*.

Some F0 1000mg/kg-day NTO-exposed birds exhibited germ cells sloughed in the lumen of seminiferous tubules. Of note, peripubertal male rats normally have intraluminal sloughed cells (especially in the epididymis) which decline in number and are no longer observed in mature rats (personal observation). The finding of sloughed germ cells may, therefore, simply reflect the immaturity of the birds, with the exception of a few observed multinucleate germ cells, which are abnormal, (Creasy, 1997, Creasy, 2001). Tubule degeneration or necrosis was not observed. Therefore, results may not be extrapolatable to the effect of NTO on adult birds. Of possible interest are the eight high- or medium-dose birds that did not exhibit developmental delays in testes. Conjecture could include dosing idiosyncrasy, regurgitation (and loss) of the dose or resistance to its effect, among others.

The bursal changes reflect either normal, physiological involution or follicular degeneration, which can be a result of stress or a test article effect. Bursal follicular cysts are associated with physiological involution. Thinning of the follicular medulla, however, with loss of the distinct junction between the cortex and medulla, indicate degeneration (EPA, 2015).

Artifacts and perimortem death:

Kidney: Kidneys from NTO-exposed F0 males and females from the two high dose groups that did not make it to scheduled sacrifice often exhibited single-cell necrosis of epithelial cells in the distal tubules. Although the renal change appears to be dose-dependent, it occurred immediately peri-mortem, based on the lack of corroborative evidence of an antemortem process. The complete absence of accompanying inflammation, regeneration, intraluminal sloughed epithelial cells, proteinuria, and casts, indicates that the renal cell changes were not associated with the primary observed pathology but occurred immediately prior to death. Interestingly, some female F0 birds but no males exhibited proximal tubule epithelial cell perimortem necrosis as well. The presence of a renal portal system differentiates avian kidneys from mammalian kidneys. It does not, however, explain the more pronounced perimortem cell death in distal tubules than in proximal tubules, which contradicts reports on avian renal autolysis (Siller, 1981). Proximal tubules, due to their abundant metabolic enzymes, are generally more sensitive to hypoxia than distal tubules. Therefore, effects in the kidneys were most likely secondary to the observed mortality and morbidity by other causes.

Liver: Most male F0 generation birds have the random fine vacuoles but only the 1000 mg/kg-day group has the centrilobular pattern that was often prominent in the F0 females. Male F1 birds (100 mg/kg-d and controls) exhibit the random but not the centrilobular hepatocellular vacuolation.

Hepatocellular vacuoles in controls fairly clearly rules out NTO as the cause but raises the question as to the origin. Processing artifacts are more likely when both the controls and exposed tissues exhibit the finding. Control tissues were deliberately processed along with each dose group, to account for processing idiosyncrasies. The fine randomly distributed hepatocellular vacuoles are presumed to be due to a processing artifact as the findings were often in control animals. Similar vacuoles were reported in the EPA Avian Two-generation toxicity test guidelines (EPA, 2015).

The cause of the centrilobular macrovesicular pattern of hepatocellular vacuolation in this study is unclear. The pattern was striking at times and present in controls as well as treated birds. The portal bridging and oval cell hyperplasia can be secondary to the vacuolation. Anoxia associated with carbon dioxide euthanasia has been reported to induce postmortem hepatocyte vacuolation when animals are not immediately bled or necropsied (Li, et al, 2003). For this study, animals euthanized on the scheduled day were necropsied within minutes of euthanasia. Animals euthanized early due to morbidity may have experienced slight delay between euthanasia and tissue fixation. The centrilobular pattern suggests a cell-specific response, more pronounced in hepatocytes bordering the central vein, which are known to receive less oxygenated blood. It is also possible that birds exhibit differential metabolism of the corn oil vehicle as centrilobular hepatocytes contain the greater proportion of cytochrome p450 enzymes. Hepatocellular vacuoles in rats were reported to contain plasma (Li et al, 2003). Oil Red O or PAS staining may help identify the contents of the vacuoles in this study.

In conclusion, oral exposures of NTO at 1000 and 500 mg/kg-d in *C. japonica* was lethal at concentrations exceeding 500 mg/kg-d. Adverse effects were not consistently observed in birds at lower concentrations and adverse effect to the male reproductive organs cannot be ruled out at lethal exposure levels. Effect on female reproductive development was not evaluable; however, is not expected as F0's produced viable offspring at daily exposures of 100 mg/kg-d and lower.

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APPENDIX A PHOTOMICROGRAPHS

Figure 1. Control quail testes. The 31-day-old Male F0 control (#293) quail has immature testes without tubule lumina (A). Compare to an 86-day old control quail (#294) with developed seminiferous tubules (B). Both photos at 2X.

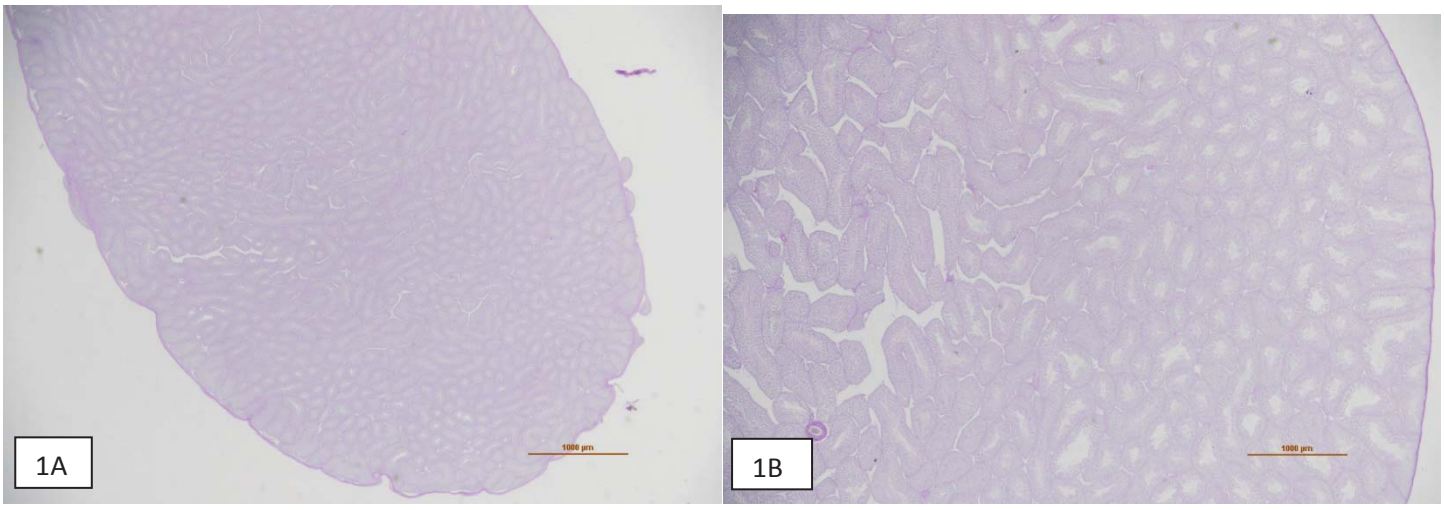
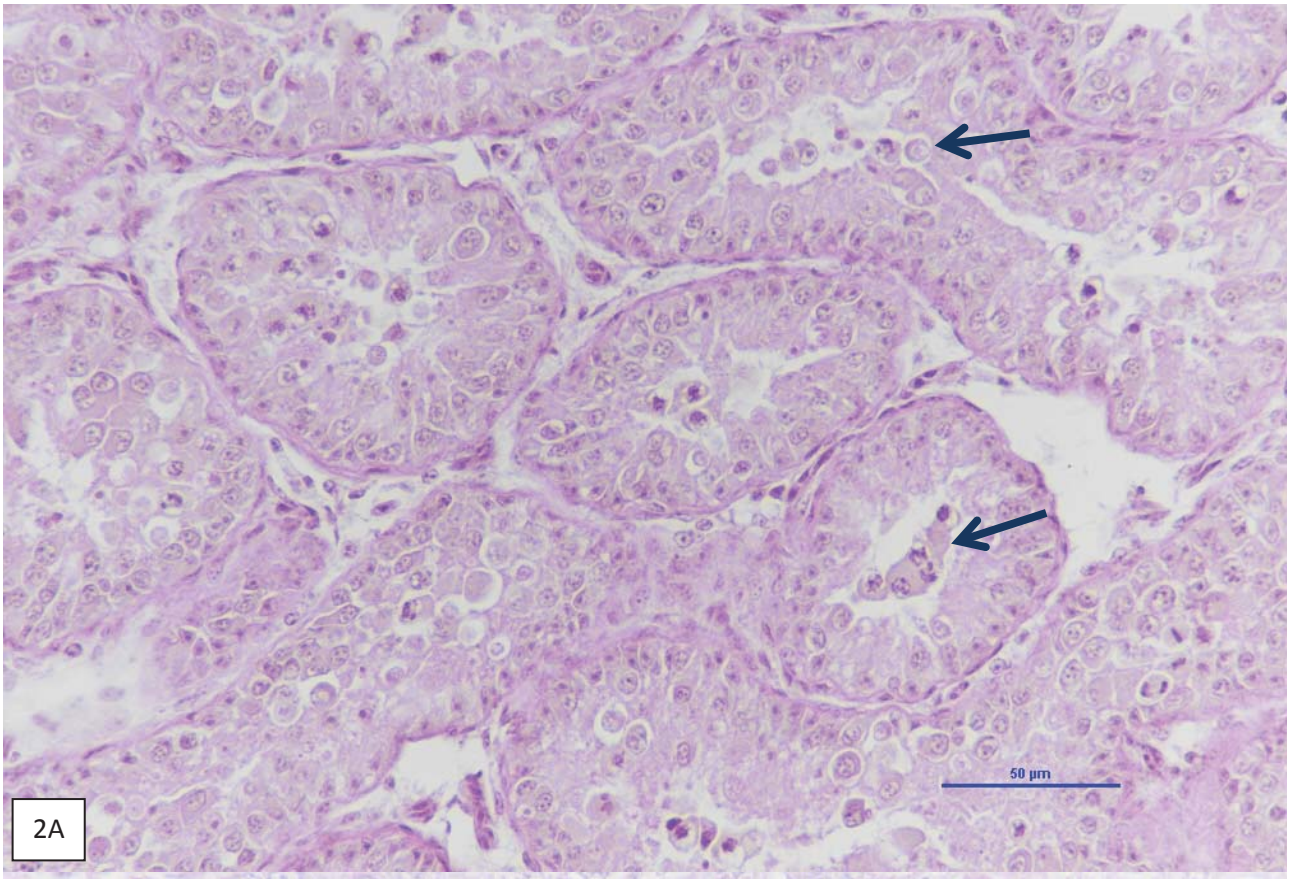
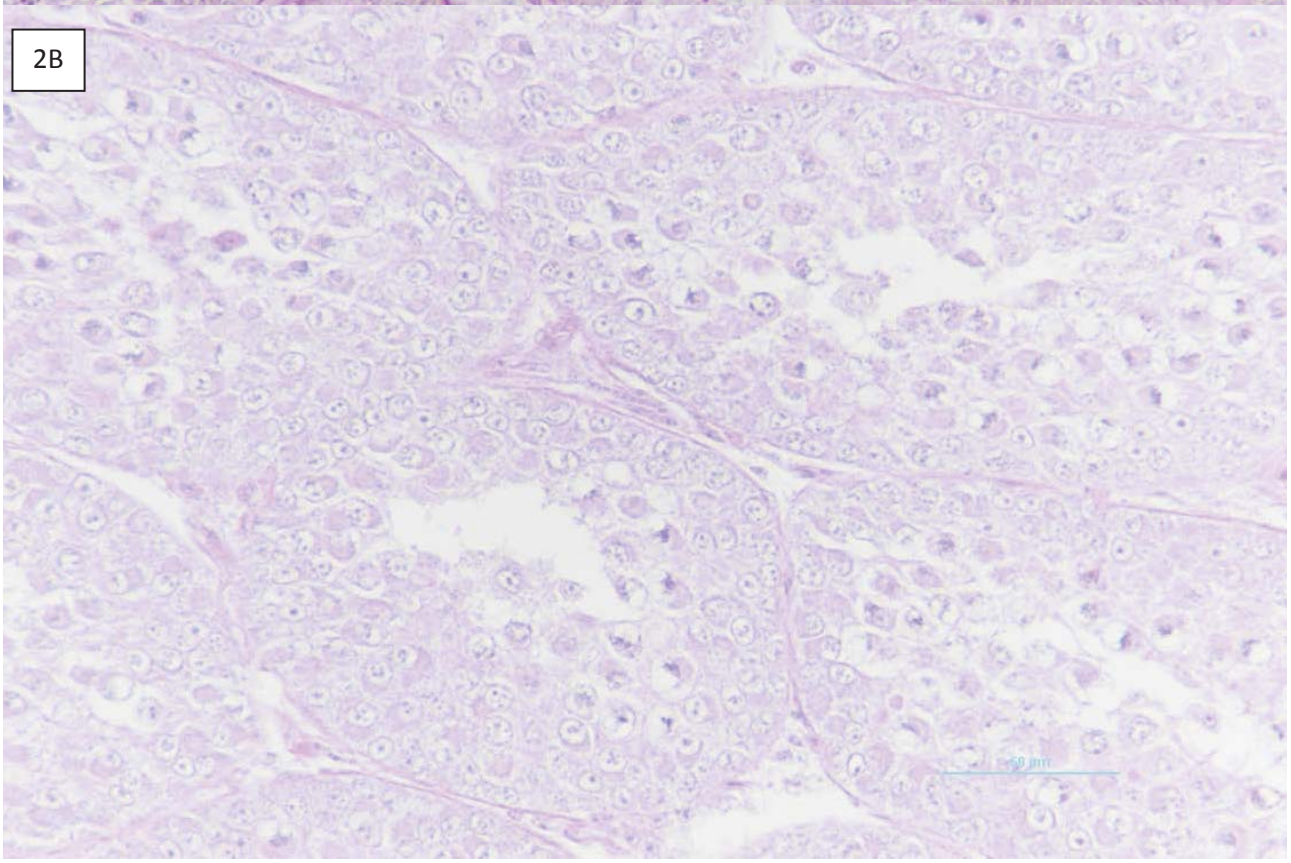


Figure 2. Sloughed degenerate or multinucleate germ cells (arrows) into seminiferous tubule lumina of a 35-day old 1000 mg/kg-day NTO-exposed quail (#366, 2A). Compare with same magnification of a 31-day old control (2B) PAS (40X).



2A



2B

Figure 3. Single cell necrosis (arrow) in NTO-exposed (and some female control) quail kidneys is perimortem. Birds have been dosed for weeks but there is no physiological response. This 500 mg/kg NTO-exposed mail quail, euthanized at 47 days old, has typical distal tubule condensation of nuclei. HE (40X)

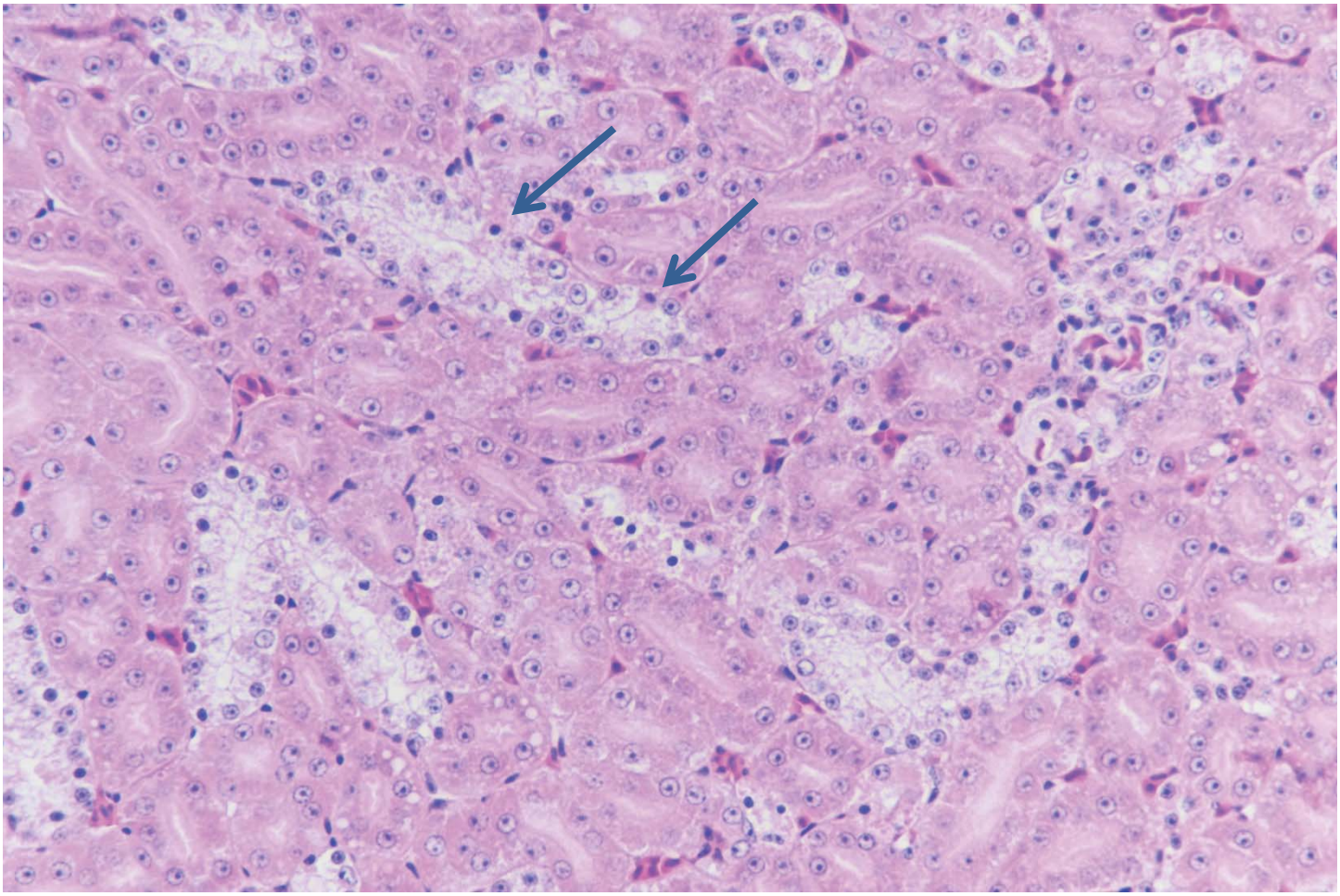


Figure 4. Bursal follicular degeneration was fairly common in NTO-exposed F1 males and F0 females. This 44-day old female F0 500 mg/kg-day NTO-exposed quail (#442, 4A) exhibited paucicellular follicles with loss of corticomedullary junction. Compare with bursa from an 86-day-old control female (arrowheads outline normal junction) (#387, 4B). 20X

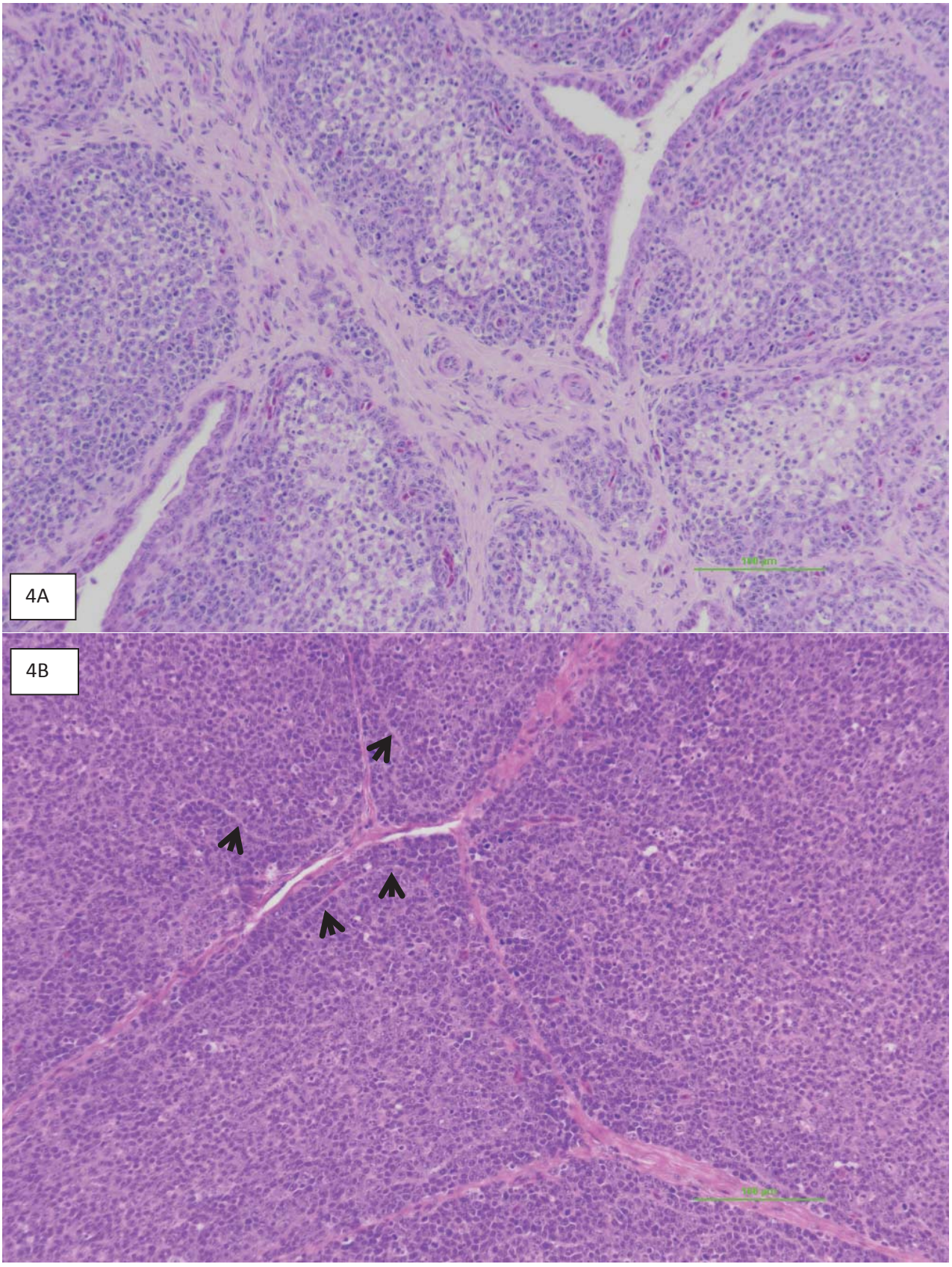
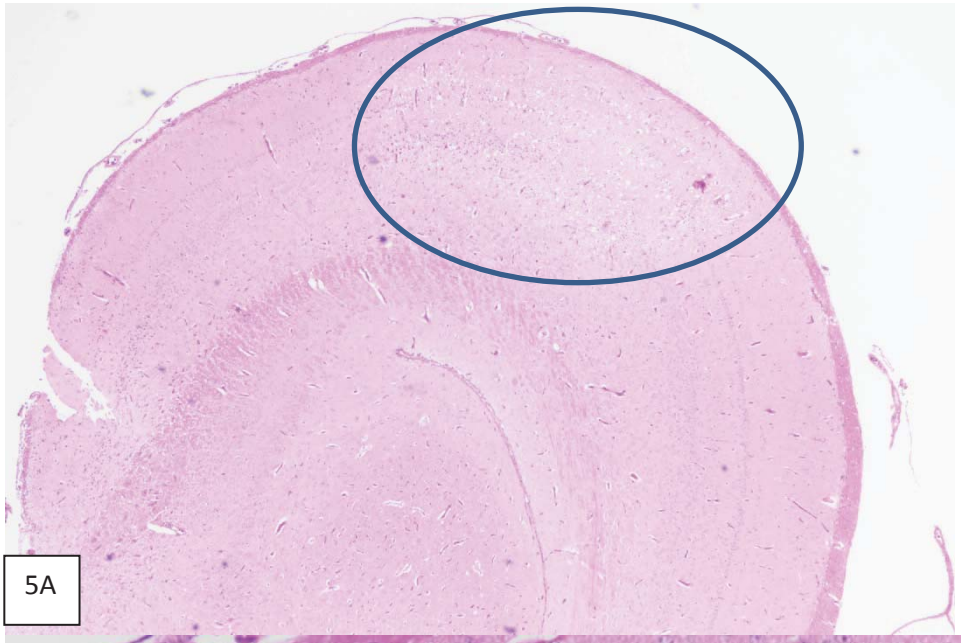
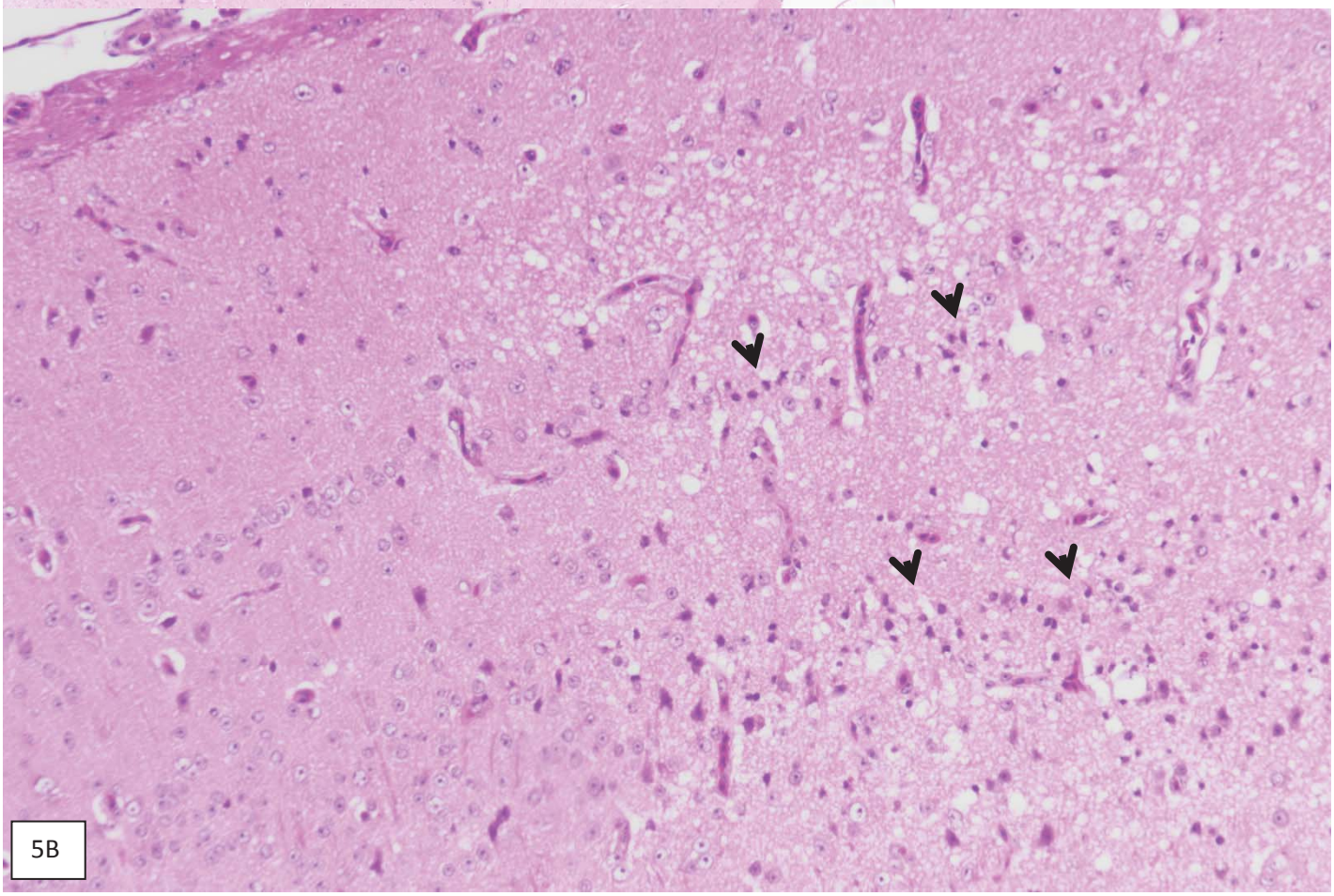


Figure 5. This focal hyperstriatal (presumptive) lesion consisting of neuropil attenuation (outlined) in a 1000 mg/kg-day NTO-exposed F0 female (#452) most likely represents injury related to thrashing in the cage (5A) HE 4X. At 20X (5B) minimal capillary congestion, rare neurononecrosis (arrowheads) and gliosis is present. Compare with more normal tissue on the left side of the image.



5A



5B

Figure 6. Six of twelve 500 mg/kg-day NTO-exposed F0 female quail exhibited single cell death in renal proximal tubules. Without physiological response, the death is most likely perimortem. This is bird #439, the caudal section of kidney. Proximal tubules, distinguished by the brush border (arrowheads), have occasional karyorrhectic nuclei (arrows). HE (40X).

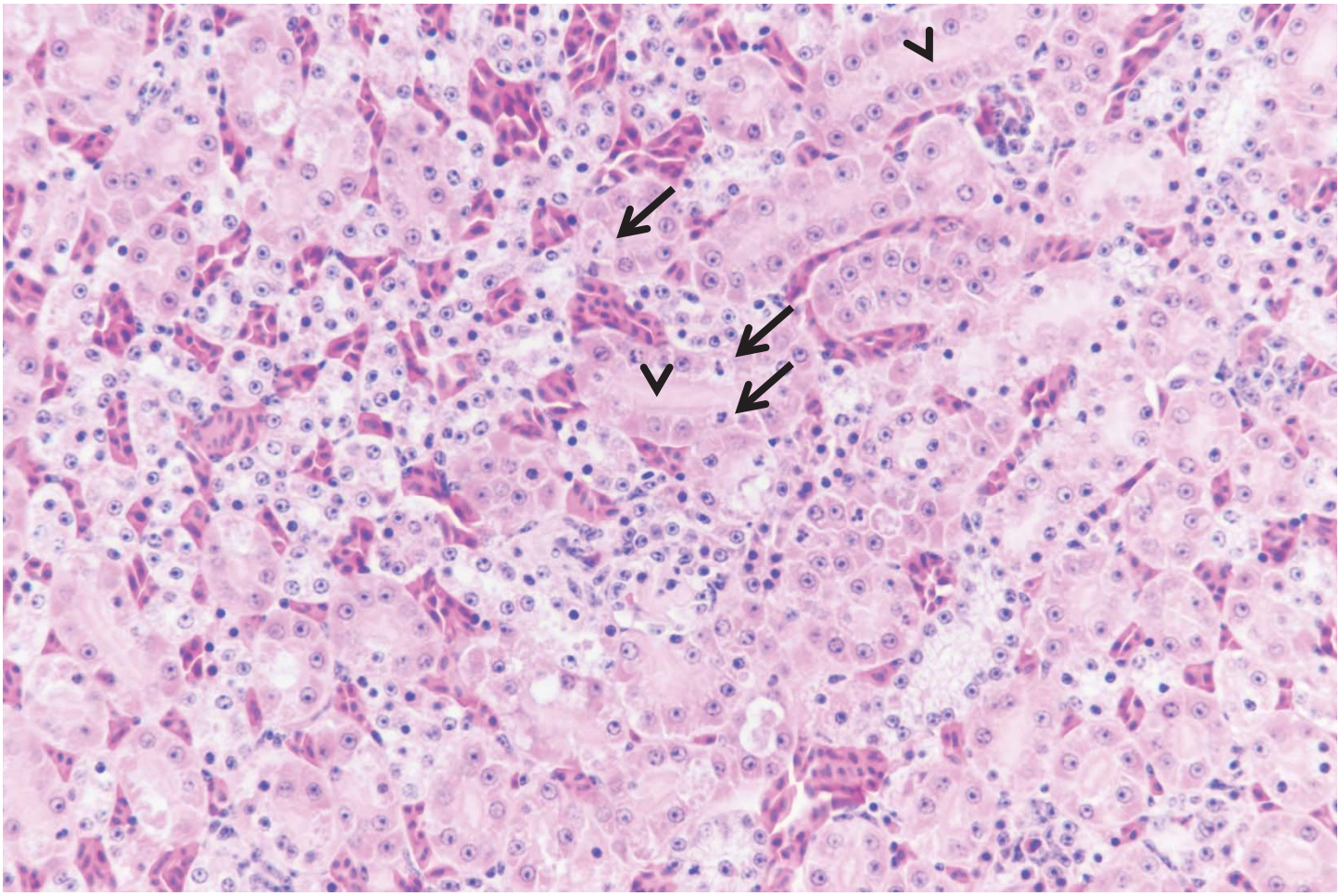


Figure 7. Ovaries of 1000 mg/kg-day NTO-exposed quail were substantially smaller than ovaries of control birds but they were also considerably younger. This high-dose F0 bird (#460, 4A) was 40 days old at euthanasia. The control was 86 days old and did not fit in the field of view (#394). Both at 2X

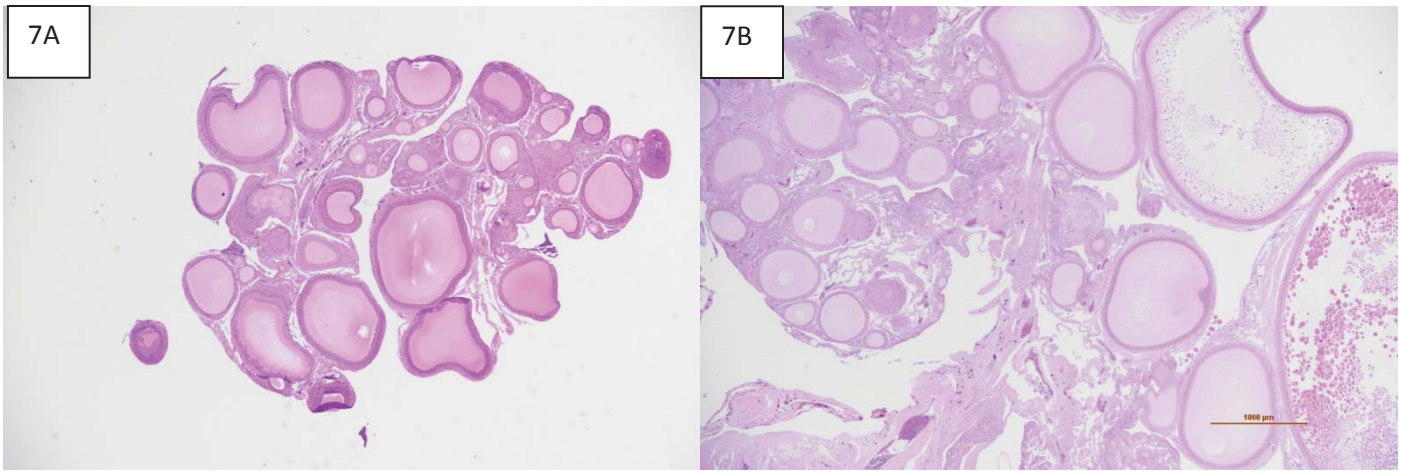


Figure 8. Numerous quail exhibited hepatocellular centrilobular macrovesicular vacuolation. This F1 control female (#691) was among the more seriously affected. HE 4X

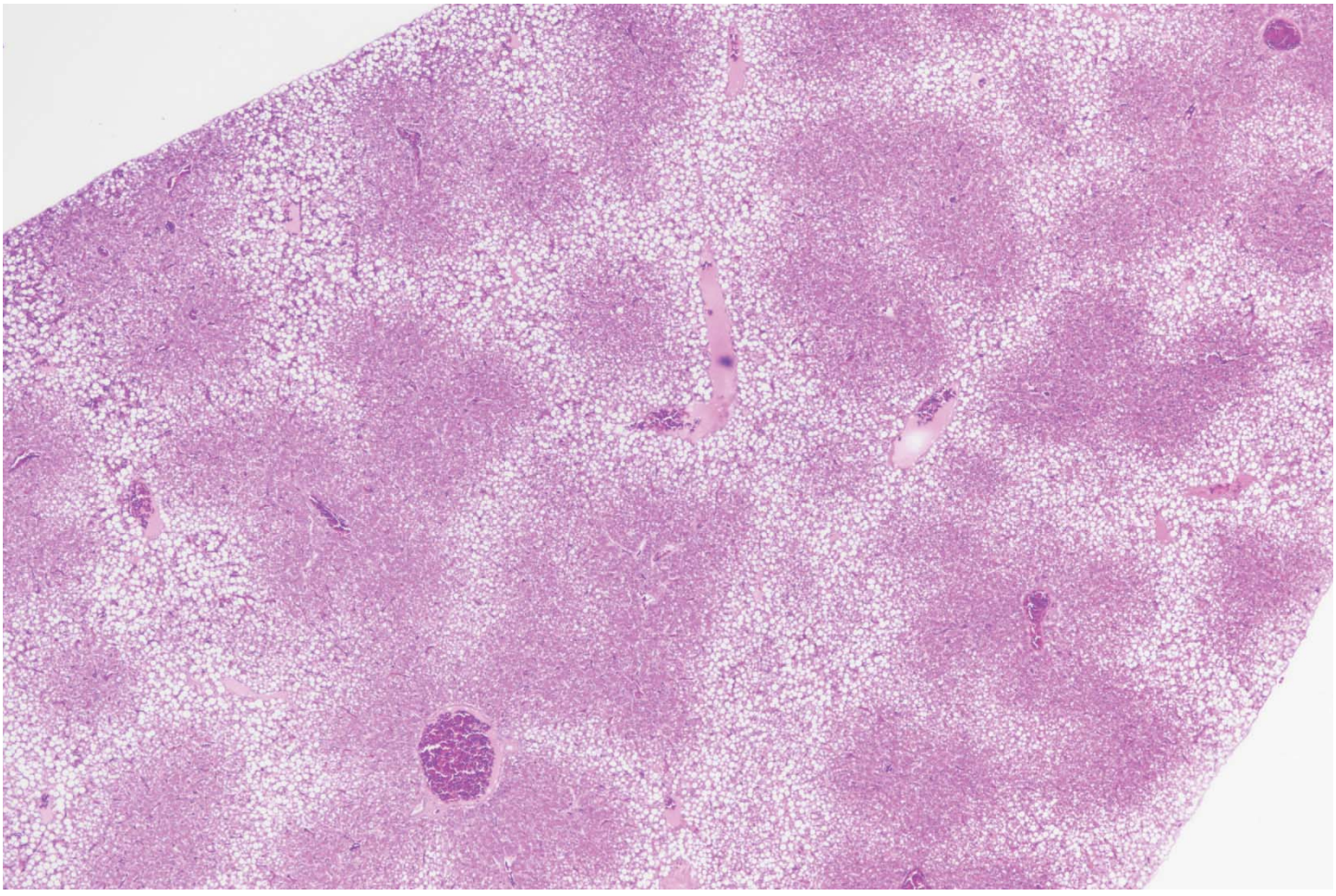
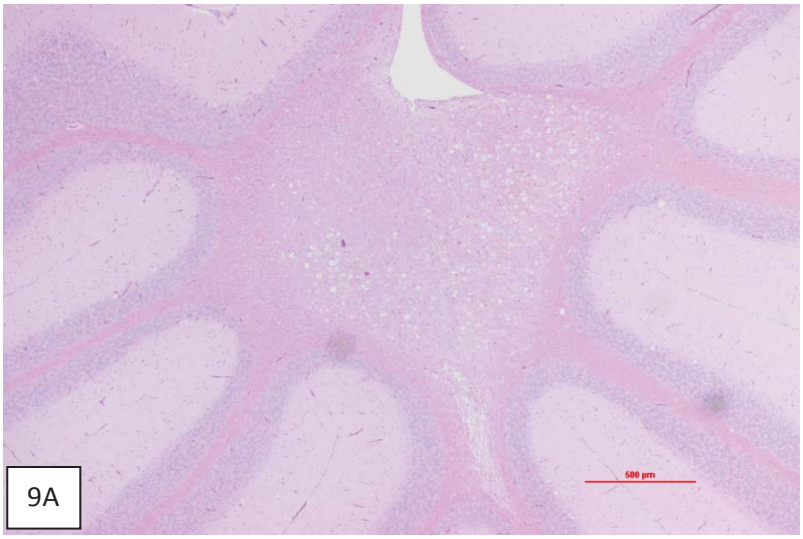
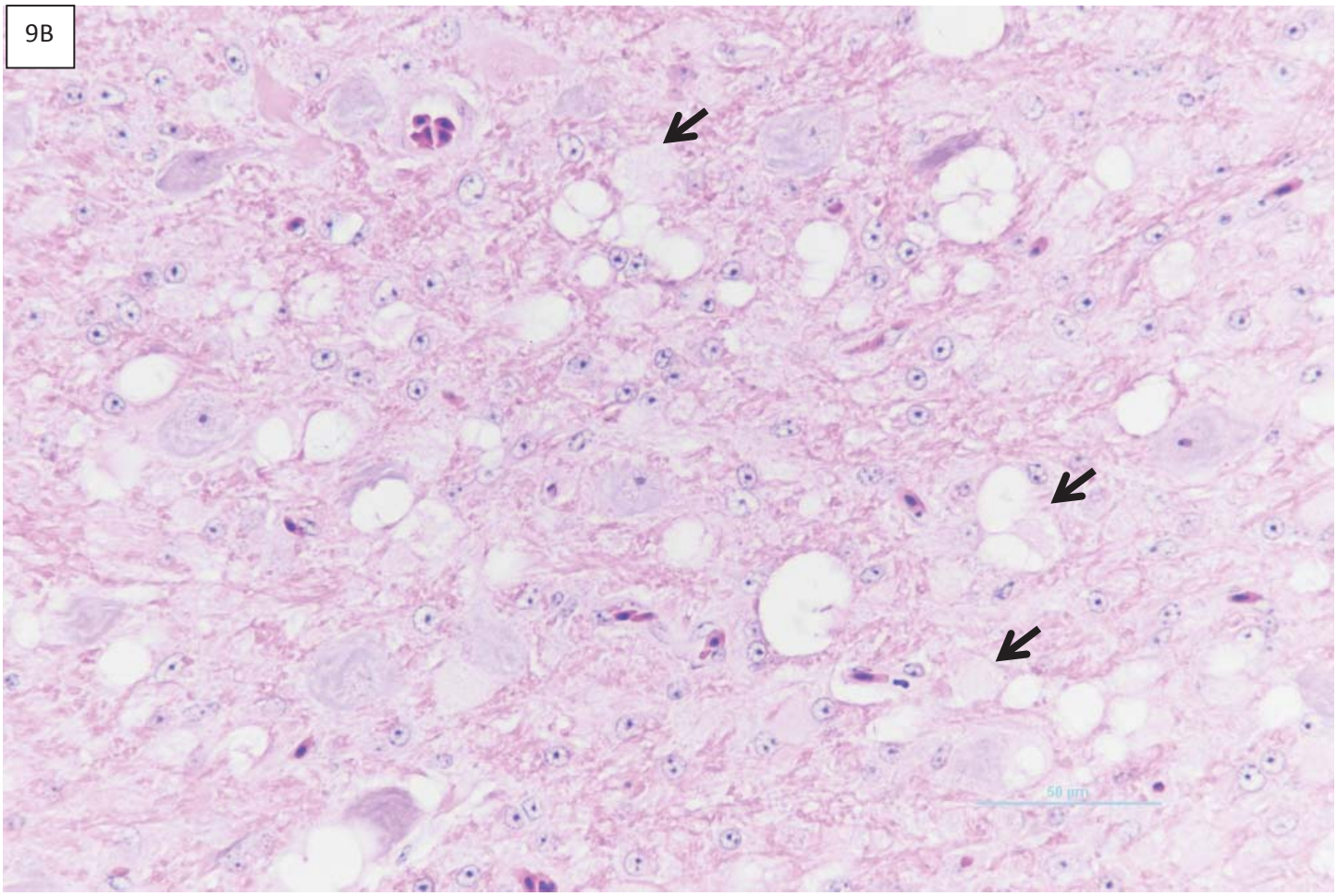


Figure 9. Cerebellar deep nuclei are mildly vacuolated in this 100mg/kg-day NTO-exposed F0 male quail (#368, 4X 9A). Vacuoles are irregularly shaped and a few contain pale eosinophilic fluid (mucocytes/Buscaino bodies, an artifact(arrows)) adjacent to apparently normal neurons (9B). HE 40X





APPENDIX B SUMMARY INCIDENCE TABLES

PARENTAL GENERATION MALES:

PARENTAL GENERATION mg/kg-d NTO-->	Controls						1000 mg/kg-day NTO						5000 mg/kg-day NTO						100 mg/kg-day NTO					
	Normal	Minimal	Mild	Moderate	Marked	Total	Normal	Minimal	Mild	Moderate	Marked	Total	Normal	Minimal	Mild	Moderate	Marked	Total	Normal	Minimal	Mild	Moderate	Marked	Total
Severity Score-->	0	1	2	3	4	Evaluated	0	1	2	3	4	Evaluated	0	1	2	3	4	Evaluated	0	1	2	3	4	Evaluated
Brain -cerebellum																								
Neuropil vacuoles-deep cerebellar white or gray (artifact)	11					11	3	8	3			14	2	11	1			14						
Liver																								
Vacuolation, random	2	10				12	10	10				20	9	5	4			18						
Vacuolation, centrilobular-midzonal	12					12	14	5		1		20	15	2	1			18						
Infiltrate, lymphoplasmacytic	10	2				12	19	1				20	16	1	1			18						
Congestion	8	4				12	13	6	1			20	9	8	1			18						
Kidney, cranial division, Spinal cord, nerve ganglion, skeletal muscle, bone & marrow +/- lung, adrenal gland																								
Distal tubules, single cell necrosis (perimortem)	10	2				12	12		3	1		16	17					17	9	3				12
Infiltrate, lymphoplasmacytic	12					12	16					16	15	2				17	11	1				12
Kidney, middle division, Spinal cord, skeletal muscle, bone & marrow																								
Distal tubules, single cell necrosis (perimortem)	12					12	10	1	3	1		15*	17	1				18	9	2				11
Feathered skin, erosion with heterophilic dermal infiltrate	11			1		12	16					16	18					18	11					11
Adipose tissue, Infiltrate, lymphoplasmacytic	10	2				12	16					16	17	1				18	9	2				11
Kidney, caudal division, Spinal cord, skeletal muscle, bone & marrow																								
Distal tubules, single cell necrosis (perimortem)	12					12	9	1	3	1	2	16	13	5				18	9	2				11
Tubule interstitiium, infiltrate, lymphocytic	12					12	14	2				16	16		2			18	11					11
Heart																								
Pigment, myocardial (mineral, presumptive)	11					11	20					20	17			1		18						
Spleen and Bursa																								
Bursa, follicular cysts	3	6	2			11	19					19	9	4	3	1		17						
Bursa, lymphocytolysis/increased tingible body macrophages	11					11	18		1			19	14	2		1		17						
Epididymis																								
Reduced sperm number in epididymis(for age)	11					11	1					1	2					2	10	1				11
Testis																								
Small size	11					11	3	3	1	1	10	18	5	2	3	4	4	18	11					11
Absence of elongating spermatids	11					11	4		1		9	14**	7	4	2	3	2	18	11					11
Vacuoles, Sertoli cells	9	2				11	15	1	1	1		18	13	5				18	10	1				11
Sloughed germ cells or multinucleate cells	11					11	10	1	1	1	5	18	14	3	1			18	10	1				11
Scoring criteria: 0='zero to <1 % of tissue is affected; 1 = < 5% of tissue is affected (minimal); 2= 6-15% of tissue is affected (mild); 3= 16-40% of tissue is affected (moderate); 4= >41% of tissue is affected (marked).																								

* = One or more tissues were unevaluable

** = The testis was too immature to have elongating spermatids in some cases. Only those old enough were evaluated for this metric.

FIRST FILIAL GENERATION (F1) MALES:

NTO-EXPOSED MALE JAPANESE QUAIL												
FIRST FILIAL GENERATION mg/kg-d NTO-->	Controls						100 mg/kg-day NTO					
	Normal	Minimal	Mild	Moderate	Marked	Total	Normal	Minimal	Mild	Moderate	Marked	Total
Severity Score-->	0	1	2	3	4	Evaluated	0	1	2	3	4	Evaluated
Brain -cerebellum												
Neuropil vacuoles-deep cerebellar white or gray (artifact)	12	3				15	16					16
Liver												
Vacuolation, random	4	8	3			15	6	10				16
Vacuolation, centrilobular-midzonal	14	1				15	14	2				16
Infiltrate, lymphoplasmacytic	8	7				15	11	5				16
Congestion	14	1				15	16					16
Kidney, cranial division, Spinal cord, nerve ganglion, skeletal muscle, bone & marrow +/- lung, adrenal gland												
Distal tubules, single cell necrosis (perimortem)	14	1				15	15					15
Infiltrate, lymphoplasmacytic	13	2				15	14	1				15
Kidney, middle division, Spinal cord, skeletal muscle, bone & marrow												
Distal tubules, single cell necrosis (perimortem)	14	1				15	15					15
Feathered skin, erosion with heterophilic dermal infiltrate	15					15	15					15
Adipose tissue, Infiltrate, lymphoplasmacytic	14	1				15	14	1				15
Kidney, caudal division, Spinal cord, skeletal muscle, bone & marrow												
Distal tubules, single cell necrosis (perimortem)	15					15	15	1				16
Tubule interstitiium, infiltrate, lymphocytic	15					15	14	2				16
Heart												
Pigment, myocardial (mineral)	15					15	16					16
Spleen and Bursa												
Bursa, follicular cysts	4	6	3	1	1	15	6	7	2	1		16
Bursa, lymphocytolysis/increased tingible body macrophages	13	2				15	10	6				16
Epididymis												
Reduced sperm number in epididymis(for age)	15					15	16					16
Testis												
Small size	14					14*	14					14*
Absence of elongating spermatids	15					15	16					16
Vacuoles, Sertoli cells	12	2	1			15	15	1				16
Sloughed germ cells or multinucleate cells	14	1				15	15	1				16

Scoring criteria: 0 ='zero to <1 % of tissue is affected; 1 = < 5% of tissue is affected (minimal); 2= 6-15% of tissue is affected (mild); 3= 16-40% of tissue is affected (moderate); 4= >41% of tissue is affected (marked).

* = One or more tissues were unevaluable and therefore not counted.

** = The testis was too immature to have elongating spermatids in a few cases, which were not included.

PARENTAL GENERATION FEMALES:

NTO-EXPOSED FEMALE JAPANESE QUAIL																								
Parental Generation (F0) Dosage in mg/kg NTO-->	Controls						1000 mg/kg-day NTO						5000 mg/kg-day NTO						100 mg/kg-day NTO					
	Normal	Minimal	Mild	Moderate	Marked	Total	Normal	Minimal	Mild	Moderate	Marked	Total	Normal	Minimal	Mild	Moderate	Marked	Total	Normal	Minimal	Mild	Moderate	Marked	Total
Severity Score-->	0	1	2	3	4	Evaluated	0	1	2	3	4	Evaluated	0	1	2	3	4	Evaluate	0	1	2	3	4	Evaluated
Liver																								
Congestion	11					11	10		1			11	6	3	3			12	11		1			12
Infiltrate, lymphoplasmacytic	8	3				11	9	2				11	12					12	9	2		1		12
Infiltrate, portal, heterophilic	11					11	11					11	12					12	11			1		12
Hyperplasia, oval cell, portal, bridging	11					11	11					11	12					12	11			1		12
Vacuolation, diffuse, random	3	4	2	1	1	11	1	5	4	1		11	11		1			12	8	2		2		12
Vacuolation, centrilobular	4	4	2		1	11	11					11	12					12	11		1			12
CRANIAL KIDNEY, Spinal cord, skeletal muscle, bone																								
Proximal Renal tubule Degeneration (loss of	11					11	10					10	9				3	12	12					12
Distal tubules, single cell necrosis (perimortem)	11					11	9	1				10	10		2			12	12					12
Tubular protein, increased	10	1				11	10					10	11	1				12	12					12
Infiltrate, interstitial, lymphoplasmacytic	9	2				11	10					10	12					12	10	1	1			12
MIDDLE KIDNEY, Spinal cord, skeletal muscle, bone																								
Proximal Renal tubule Degeneration	11					11	9	1				10	7	2	1		2	12	12					12
Distal tubules, single cell necrosis (perimortem)	11					11	10					10	9	1	2			12	11	1				12
Skeletal muscle: Myositis, lymphohistiocytic and	11					11	10					10	11		1			12	12					12
Tubular protein, increased	10	1				11	10					10	11	1				12	12					12
Infiltrate, interstitial, heterophilic	10	1				11	10					10	12					12	12					12
Infiltrate, interstitial, lymphoplasmacytic	11					11	10					10	12					12	10	2				12
CAUDAL KIDNEY, Spinal cord, skeletal muscle, bone																								
Proximal Renal tubule Degeneration	10	1				11	9	1				10	6	2	1	1	2	12	12					12
Distal tubules, single cell necrosis (perimortem)	9	2				11	7	3				10	10	1	1			12	11	1				12
Infiltrate, interstitial, lymphoplasmacytic	9	2				11	8	2				10	12					12	9	3				12
Heart																								
Myocarditis, subacute	10					10	10			1		11	12					12	12					12
Pigment, myocardial (mineral)	10					10	9	2				11	12					12	12					12
Pericaridal fat, infiltrate, histiocytic	10					10	10		1			11	12					12	12					12
Spleen and Bursa																								
Bursa, follicular cysts	3	3	3	1		10	7	1	1			9	10	2				12	9	3				12
Bursa, lymphocytolysis or increased tingible body macrophages	9	1				10	5	4				9	7	3	1	1		12	9	3				12
Thyroid gland (occ with thymus)																								
Infiltrate, lymphoplasmacytic	11					11	8					8	10		1			12	7	2				9
Infundibulum, Magnum												0	2					2						
Infiltrate, lymphoplasmacytic	10	1				11													8	4				12
Ovary, left																								
Immature	11					11			2	2	6	10	3		1	6	2	12	12					12
Isthmus																								
Infiltrate, lymphoplasmacytic	10	1				11						0	2					2	11		1			12
Shell gland (Uterus)/vagina	11					11						0	2					2						

Scoring criteria: 0 ='zero to <1 % of tissue is affected; 1 = < 5% of tissue is affected (minimal); 2= 6-15% of tissue is affected (mild); 3= 16-40% of tissue is affected (moderate); 4= >41% of tissue is affected (marked).

FIRST FILIAL GENERATION (F1) FEMALES

NTO-EXPOSED FEMALE JAPANESE QUAIL												
Dosage in mg/kg NTO-->	Controls						100 mg/kg-day NTO					
	Normal	Minimal	Mild	Moderate	Marked	Total	Normal	Minimal	Mild	Moderate	Marked	Total
Severity Score-->	0	1	2	3	4	Evaluate	0	1	2	3	4	Evaluate
Liver												
Congestion	13					13	13	2				15
Infiltrate, lymphoplasmacytic	7	6				13	10	3	2			15
Infiltrate, portal, heterophilic	13					13	14	1				15
Hyperplasia, oval cell, portal, bridging	12	1				13	12	1	1	1		15
Vacuolation, diffuse, random	6	5	2			13	8	4	2	1		15
Vacuolation, centrilobular	5	5		2	1	13	7	3	2	3		15
CRANIAL KIDNEY, Spinal cord, skeletal muscle, bone												
Proximal Renal tubule Degeneration (perimortem)	11	1	1			13	15					15
Distal tubules, single cell necrosis (perimortem)	11	1	1			13	14	1				15
Tubular protein, increased	12	1				13	15					15
Infiltrate, interstitial, lymphoplasmacytic	11	2				13	14	1				15
MIDDLE KIDNEY, Spinal cord, skeletal muscle, bone												
Proximal Renal tubule Degeneration	12		1			13	15					15
Distal tubules, single cell necrosis (perimortem)	12		1			13	15					15
Skeletal muscle: Myositis, lymphohistiocytic and	13					13	15					15
Tubular protein, increased	12	1				13	15					15
Infiltrate, interstitial, heterophilic	13					13	15					15
Infiltrate, interstitial, lymphoplasmacytic	13					13	14	1				15
CAUDAL KIDNEY, Spinal cord, skeletal muscle, bone												
Proximal Renal tubule Degeneration	12		1			13	15					15
Distal tubules, single cell necrosis (perimortem)	12			1		13	14		1			15
Infiltrate, interstitial, lymphoplasmacytic	9	4				13	13	1		1		15
Heart												
Myocarditis, subacute	13					13	15					15
Pigment, myocardial (mineral)	13					13	15					15
Pericaridal fat, infiltrate, histiocytic	13					13	15					15
Spleen and Bursa												
Bursa, follicular cysts	7	4	1	1		13	5	6	1	1		13
Bursa, lymphocytolysis or increased tingible body macrophages	12	1				13	12	1				13
Thyroid gland (occ with thymus)												
Infiltrate, lymphoplasmacytic	11	1				12	12	3				15
Infundibulum, Magnum												
Infiltrate, lymphoplasmacytic	11	2				13	14		1			15
Ovary, left												
Immature	13					13	15					15
Isthmus												
Infiltrate, lymphoplasmacytic	10	1				11	12	3				15
Shell gland (Uterus)/vagina	12					12	15					15

Scoring criteria: 0 ='zero to <1 % of tissue is affected; 1 = < 5% of tissue is affected (minimal); 2= 6-15% of tissue is affected (mild); 3= 16-40% of tissue is affected (moderate); 4= >41% of tissue is affected (marked).

APPENDIX C INDIVIDUAL ANIMAL DATA

Controls quail were compared to 1000 , 500, and 100 mg/kg-day-NTO exposed quail.

Scoring criteria: 0 ='zero to <1 % of tissue is affected; 1 = < 5% of tissue is affected (minimal); 2= 6-15% of tissue is affected (mild); 3= 16-40% of tissue is affected (moderate); 4= >41% of tissue is affected (marked).

After evaluation of controls and high-dose male F0 birds, test article-related lesions were only suspected in kidneys and reproductive tissues; therefore, only those tissues were evaluated in 100 mg/kg-day-exposed F0 males. P = Present. NP = Not Present.

Parental Generation (F0) Males

All Identification numbers are prefaced with '15-'	284	285	286	287	288	289	291	292	293	294	296	297	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385
MALE Dosage in mg/kg NTO-->	0	0	0	0	0	0	0	0	0	0	0	0	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Hatched 23 FEB 15 Date of euthanasia-->	5/20	5/20	5/20	5/20	5/20	5/20	5/20	5/20	3/26	5/20	5/20	5/20	3/29	3/30	4/3	4/3	3/26	3/29	3/26	3/28	4/1	3/30	3/25	3/28	3/27	3/25	3/31	3/30	3/28	4/1	4/1	4/1
Slide #. Tissue AGE in days-->	86	86	86	86	86	86	86	86	31	86	86	86	34	35	39	39	31	34	31	33	37	35	30	33	32	30	36	35	33	37	37	37
1. Brain - cerebrum-medial preoptic nucleus	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	NP	P	P	P	NP	P	P	NP	P	P	P	NP	P	P
2. Brain -cerebellum	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	NP	P	NP	P	P	P	NP	P	NP	NP	P	P	P	NP	P	P
Neuropil vacuoles-deep cerebellar white or gray (artifact)	0	0	0	0	0	.	0	0	0	0	0	0	2	0	2	1	.	1	.	1	1	1	.	1	.	.	0	2	1	.	0	1
3. Liver	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Vacuolation, random	1	1	1	1	0	1	1	1	0	1	1	1	0	0	0	0	1	0	0	0	1	0	0	0	1	1	1	1	1	1	1	1
Vacuolation, centrilobular-midzonal	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	0	0	0
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Congestion	1	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0	1	1	1	2	0	0	0	0	0
4. Kidney, cranial division, Spinal cord, nerve ganglion, skeletal muscle, bone with marrow +/-lung, adrenal gland	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	NP	P	P	NP	P	P	P	NP	P	P
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	1	0	0	0	0	1	0	0	2	0	0	0	.	0	0	2	0	0	.	3	0	.	0	0	2	.	0	0
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	0	0	0	.	0	0	.	0	0	0	.	0	0
5. Kidney, middle division, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	NP	P	P	P	NP	NP	P
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	3	0	0	.	2	NE	.	0	0	1	.	.	0
Feathered skin, erosion with heterophilic dermal infiltrate	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	.	0	0	0	.	.	0
Adipose tissue, Infiltrate, lymphoplasmacytic	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	.	0	0	0	.	.	0
6. Kidney, caudal division, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	NP	P	P	NP	P	P	P	NP	P	P
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	4	0	2	0	0	.	0	3	0	0	.	4	2	.	1	0	2	.	0	0
Tubule interstitiium, infiltrate, lymphocytic	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	.	0	0	0	0	.	0	0	.	0	0	0	.	0	0
7. Heart	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Pigment, myocardial (mineral, presumptive)	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8. Spleen and Bursa	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Bursa, follicular cysts	0	1	2	1	1	0	.	2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	NP	0	0	0	0	0	0	0
Bursa, lymphocytolysis/increased tingible body macrophages (involution or degeneration	0	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NP	0	0	0	0	2	0	0
9. Thyroid gland	P	P	P	P	P	NP	P	P	NP	P	P	P	P	P	P	NP	NP	P	P	P	P	NP	P	P	P	P	P	P	P	NP	P	P
10. Cloacal glands, +/- large intestine,	P	P	P	P	P	P	P	P	NP	P	P	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Cloacal gl, lamina propria, lymphoplasmacytic (normal)	1	1	2	0	1	2	0	0	.	0	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Epididymis	P	P	P	P	P	NP	P	P	P	P	P	P	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Reduced sperm number in epididymis(for age)	0	0	0	0	0	.	0	0	0	0	0	0	.	.	.	.	.	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
12. Testis	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	NP	P	P	P	P	P	P
Small size	0	0	0	0	0	.	0	0	0	0	0	0	4	4	0	4	2	0	.	4	1	1	0	4	4	.	4	4	4	4	3	1
Absence of elongating spermatids	0	0	0	0	0	.	0	0	0	0	0	0	NE	NE	0	4	NE	0	.	NE	4	2	0	4	0	.	4	4	4	4	4	4
Vacuoles, Sertoli cells	0	1	0	1	0	.	0	0	0	0	0	0	0	0	0	0	3	0	.	0	0	0	0	0	0	.	0	0	0	0	2	1
Sloughed germ cells or multinucleate cells	0	0	0	0	0	.	0	0	0	0	0	0	3	2	0	4	4	0	.	4	0	0	1	4	4	.	0	0	0	0	0	0

All Identification numbers are prefaced with '15-'	284	285	286	287	288	289	291	292	293	294	296	297	344	346	347	348	350	351	352	353	354	355	356	357	358	359	360	362	363	364	365
MALE Dosage in mg/kg NTO-->	0	0	0	0	0	0	0	0	0	0	0	0	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500
Hatched 23 FEB 15 **Date of euthanasia-->**	5/20	5/20	5/20	5/20	5/20	5/20	5/20	5/20	3/26	5/20	5/20	5/20	4/27	4/10	4/6	5/19	4/6	4/20	4/6	4/10	4/6	4/10	4/2	4/6	4/3	4/10	4/8	5/12	4/1	3/27	3/26
Slide #. Tissue AGE in days-->	86	86	86	86	86	86	86	86	31	86	86	86	64	47	43	86	43	57	43	47	43	47	39	43	40	47	45	79	38	33	32
1. Brain - cerebrum-medial preoptic nucleus	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	NP	NP	P	P	P	P	P	P	P	P	P	P	P	P	NP
2. Brain -cerebellum	P	P	P	P	P	NP	P	P	P	P	P	P	NP	P	P	P	NP	NP	P	P	NP	P	P	P	P	P	P	P	P	P	NP
Neuropil vacuoles-deep cerebellar white or gray (artifact)	0	0	0	0	0	.	0	0	0	0	0	0	.	1	1	0	.	.	1	1	.	1	1	1	2	1	1	1	0	1	.
3. Liver	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Vacuolation, random	1	1	1	1	0	1	1	1	0	1	1	1	0	0	0	1	.	1	2	0	1	0	1	0	1	0	0	0	2	2	2
Vacuolation, centrilobular-midzonal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	0	0	0	1	1	0	0	0	2	0	0	0
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	.	0	0	0	0	0	1	0	0	2	0	0	0	0	0
Congestion	1	1	0	1	0	0	0	0	0	1	0	0	1	1	1	0	.	1	1	0	0	0	1	2	1	0	0	0	1	0	0
4. Kidney, cranial division, Spinal cord, nerve ganglion, skeletal muscle, bone with marrow +/-lung, adrenal gland	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	NP	P	P	P	P	P	P	P	P	P	P	P	NE	P	P
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	1	0	0	0	0	1	0	0	0	0	.	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	0	0	0	0	0	0	1	0	.	1	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5. Kidney, middle division, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Feathered skin, erosion with heterophilic dermal infiltrate	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adipose tissue, Infiltrate, lymphoplasmacytic	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	.	0	1	0	0	0	0	0	0	0	0	0	0	0	0
6. Kidney, caudal division, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	.	1	1	0	0	0	0	0	0	1	0	0	1	0	0
Tubule interstitiium, infiltrate, lymphocytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	0	0	0	0	0	2	0	0	0	2	0	0
7. Heart	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P
Pigment, myocardial (mineral, presumptive)	0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0	0	0	3	0	.	0	0	0	0	0	0	0	0	0	0
8. Spleen and Bursa	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Bursa, follicular cysts	0	1	2	1	1	0	.	2	0	1	1	1	NP	0	0	2	.	2	0	1	1	3	0	1	0	2	0	1	0	0	0
Bursa, lymphocytolysis/increased tingible body macrophages (involution or degeneration	0	0	0	0	0	0	.	0	0	0	0	0	NP	0	0	0	.	0	0	3	0	0	0	0	0	0	0	1	0	0	1
9. Thyroid gland	P	P	P	P	P	NP	P	P	NP	P	P	P	P	P	P	P	NP	P	P	P	NP	P	P	P	P	P	P	P	P	P	P
10. Cloacal glands, +/- large intestine,	P	P	P	P	P	P	P	P	NP	P	P	P	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Cloacal gl, lamina propria, lymphoplasmacytic (normal)	1	1	2	0	1	2	0	0	.	0	0	0	.	.	.	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Epididymis	P	P	P	P	P	NP	P	P	P	P	P	P	NP	NP	NP	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	P	NP	NP	NP	NP	NP
Reduced sperm number in epididymis(for age)	0	0	0	0	0	.	0	0	0	0	0	0	.	.	.	0	.	.	.	.	.	.	.	.	.	0	.	.	.	.	.
12. Testis	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P
Small size	0	0	0	0	0	.	0	0	0	0	0	0	4	3	2	3	4	3	0	0	2	0	3	.	1	0	4	0	4	2	1
Absence of elongating spermatids	0	0	0	0	0	.	0	0	0	0	0	0	1	2	1	1	4	3	0	0	2	0	3	.	1	0	4	0	3	0	0
Vacuoles, Sertoli cells	0	1	0	1	0	.	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	1	0	.	0	0	0	0	0	0	0
Sloughed germ cells or multinucleate cells	0	0	0	0	0	.	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	.	0	0	0	1	2	0	0

All Identification numbers are prefaced with '15-'		284	285	286	287	288	289	291	292	293	294	296	297	329	330	332	333	334	335	337	338	339	340	341	343
MALE	Dosage in mg/kg NTO-->	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	100	100	100	100	100	100	100	100	100
Hatched 23 FEB 15	**Date of euthanasia-->**	5/20	5/20	5/20	5/20	5/20	5/20	5/20	5/20	3/26	5/20	5/20	5/20	5/19	5/19	5/19	5/20	5/20	5/20	5/20	5/21	5/1	5/21	5/21	5/21
Slide #. Tissue	AGE in days-->	86	86	86	86	86	86	86	86	31	86	86	86	85	85	85	86	86	86	86	87	67	87	87	87
4. Kidney, cranial division, Spinal cord, nerve ganglion, skeletal muscle, bone with marrow +/-lung, adrenal gland		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Distal tubules, single cell necrosis (perimortem)		0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1
Infiltrate, lymphoplasmacytic		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
5. Kidney, middle division, Spinal cord, skeletal muscle, bone with marrow		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P
Distal tubules, single cell necrosis (perimortem)		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	.	0	0	1	0
Feathered skin, erosion with heterophilic dermal infiltrate		0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	.	0	0	0	0
Adipose tissue, Infiltrate, lymphoplasmacytic		0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	.	0	0	0	0
6. Kidney, caudal division, Spinal cord, skeletal muscle, bone with marrow		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P
Distal tubules, single cell necrosis (perimortem)		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	.	1	0	0	0	0	0
Tubule interstitiium, infiltrate, lymphocytic		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	0	0	0	0
Epididymis		P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P
Reduced sperm number in epididymis(for age)		0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	1	0
12. Testis		P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P
Small size		0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	0
Absence of elongating spermatids		0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	0
Vacuoles, Sertoli cells		0	1	0	1	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	1
Sloughed germ cells or multinucleate cells		0	0	0	0	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	1	0

First Filial Generation (F1) Males

All Identification numbers are prefaced with '15-'	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679
MALE Dosage in mg/kg NTO-->	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Hatched 23 FEB 15 **Date of euthanasia-->**	8/18	8/17	8/17	8/18	8/20	8/17	8/19	8/18	8/19	8/19	8/18	8/18	8/20	8/20	8/17	8/17	8/17	8/20	8/18	8/19	8/19	8/20	8/17	8/18	8/20	8/18	8/19	8/18	8/17	8/19	8/20
Slide #. Tissue AGE in days-->	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
1. Brain - cerebrum-medial preoptic nucleus	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2. Brain -cerebellum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Neuropil vacuoles-deep cerebellar white or gray (artifact)	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3. Liver	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Vacuolation, random	0	2	0	2	1	1	1	0	1	0	1	1	1	1	2	0	0	0	1	1	1	1	0	1	1	0	1	0	1	1	1
Vacuolation, centrilobular-midzonal	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Infiltrate, lymphoplasmacytic	0	1	1	0	1	0	0	0	1	0	1	0	0	1	1	1	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0
Congestion	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4. Kidney, cranial division, Spinal cord, nerve ganglion, skeletal muscle, bone with marrow +/-lung, adrenal gland	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, lymphoplasmacytic	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	.	0	0	0	0	0	0	0	0	1	0	0	0	0	0
5. Kidney, middle division, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	0	0	0
Feathered skin, erosion with heterophilic dermal infiltrate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	0	0	0
Adipose tissue, Infiltrate, lymphoplasmacytic	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	.	0	0	0	0	0
6. Kidney, caudal division, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Tubule interstitiium, infiltrate, lymphocytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
7. Heart	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Pigment, myocardial (mineral, presumptive)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	p	0	0	0	0	0	0	0	0	0
8. Spleen and Bursa	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Bursa, follicular cysts	1	1	2	0	1	0	2	2	0	4	1	1	1	3	0	2	1	1	1	3	1	1	0	1	2	0	0	0	0	1	0
Bursa, lymphocytolysis/increased tingible body macrophages (involution or degeneration	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	1	0	0
9. Thyroid gland	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
10. Cloacal glands, +/- large intestine,	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P
Cloacal gl, lamina propria, lymphoplasmacytic (normal)	1	1	2	2	1	1	2	.	0	0	3	1	2	0	2	0	0	0	1	0	0	0	.	1	0	0	0	0	0	0	0
Epididymis	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Reduced sperm number in epididymis(for age)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12. Testis	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Small size	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	0	NE	0	NE	0	0	0	0	0	0	0	0	0	0	0	0	0
Absence of elongating spermatids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vacuoles, Sertoli cells	0	0	1	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Sloughed germ cells or multinucleate cells	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

Parental Generation (F0) Females

FEMALE Japanese Quail ID's are prefaced with '15-'	387	388	391	392	393	394	395	396	398	399	402	451	452	454	455	456	457	458	459	460	461	462	463
Female Dosage in mg/kg NTO-->	0	0	0	0	0	0	0	0	0	0	0	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Date of euthanasia-->	5/20	5/20	5/20	5/20	5/20	5/20	5/20	5/21	5/20	5/20	5/20	3/29	3/30	3/31	3/26	4/3	3/27	3/26	3/25	4/3	3/28	3/25	4/3
Age at death (days)-->	86	86	86	86	86	86	86	87	86	86	86	35	36	37	29	40	33	32	31	40	34	31	40
1. Brain - cerebrum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	NP	P	P	NP	P
2. Brain -cerebellum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	NP	P	P	NP	P
3. Liver	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P
Congestion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	.	0
Infiltrate, lymphoplasmacytic	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	.	0
Infiltrate, portal, heterophilic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0
Hyperplasia, oval cell, portal, bridging	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0
Vacuolation, diffuse, random	1	0	2	1	0	1	3	0	1	4	2	1	2	2	0	1	1	3	2	2	1	.	1
Vacuolation, centrilobular	0	2	0	0	1	0	1	1	2	4	1	0	0	0	0	0	0	0	0	0	0		0
4. **CRANIAL KIDNEY**, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	NP	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail, karryorhexis), perimortem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	.	0
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	1	.	0
Tubular protein, increased	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	.	0	0	.	0
Infiltrate, interstitial, lymphoplasmacytic	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	.	0	0	.	0
5. **MIDDLE KIDNE**Y, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	NP	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail, karryorhexis)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	1	.	0
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	.	0
Skeletal muscle: Myositis, lymphohistiocytic and heterophilic, focal, mild, with myodegen and nec.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	.	0
Tubular protein, increased	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	.	0	0	.	0
Infiltrate, interstitial, heterophilic	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	.	0	0	.	0
Infiltrate, interstitial, lymphoplasmacytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0		0
6. CAUDAL KIDNEY, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	NP	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail with karryorhexis)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	.	0	1	.	0
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	.	0	1	.	1
Infiltrate, interstitial, lymphoplasmacytic	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	.	0	1	.	0
7. Heart	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P
Myocarditis, subacute	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	.	0
Pigment, myocardial (mineral)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	.	0
Pericaridal fat, infiltrate, histiocytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	.	0
8. Spleen and Bursa	P	P	P	P	P*	P	P	P	P	P	P	P	P	P	p	P	P	NP	P	P	P	.	P
Bursa, follicular cysts	1	2	0	0	NP	0	2	1	2	3	1	0	2	0	0	0	0	.	0	.	0	.	1
Bursa, lymphocytolysis or increased tingible body macrophages	0	0	0	0	NP	0	0	1	0	0	0	1	1	0	1	0	0	.	0	.	1	.	0
9. Thyroid gland (occ with thymus)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	NP	P	P	NP	NP	P
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.	0	0	.	0	0	.	.	0
10. Infundibulum, Magnum	P	P	P	P	P	P	P	P	P	P	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Infiltrate, lymphoplasmacytic	0	0	0	0	0	1	0	0	0	0	0	.	.	.	.	.	.	.	.	.	.	.	.
11. Ovary, left	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	.	P
Immature	0	0	0	0	0	0	0	0	0	0	0	2	3	.	4	3	2	4	4	4	4	.	4
12. Isthmus	P	P	P	P	NP	P	P	P	P	P	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	1	0	0	0	0	.	.	.	.	.	.	.	.	.	.	.	.
13. Shell gland (Uterus)/vagina	P	P	P	P	P	P	P	P	P	P	P	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP

FEMALE Japanese Quail ID's are prefaced with '15-'	387	388	391	392	393	394	395	396	398	399	402	435	438	439	440	441	442	443	445	446	447	448	450
Female Dosage in mg/kg NTO-->	0	0	0	0	0	0	0	0	0	0	0	500	500	500	500	500	500	500	500	500	500	500	500
Date of euthanasia-->	5/20	5/20	5/20	5/20	5/20	5/20	5/20	5/21	5/20	5/20	5/20	4/6	3/30	3/29	4/6	4/9	4/8	4/20	3/28	4/20	4/9	4/6	3/28
Age at death (days)-->	86	86	86	86	86	86	86	87	86	86	86	42	35	34	42	45	44	56	33	56	45	42	33
1. Brain - cerebrum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	NP	P	P	P
2. Brain -cerebellum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	NP	P	P	P
3. Liver	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Congestion	0	0	0	0	0	0	0	0	0	0	0	2	0	2	1	0	0	0	2	0	0	1	1
Infiltrate, lymphoplasmacytic	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, portal, heterophilic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hyperplasia, oval cell, portal, bridging	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vacuolation, diffuse, random	1	0	2	1	0	1	3	0	1	4	2	2	0	0	0	0	0	0	0	0	0	0	0
Vacuolation, centrilobular	0	2	0	0	1	0	1	1	2	4	1	0	0	0	0	0	0	0	0	0	0	0	0
4. **CRANIAL KIDNEY**, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail, karryorhexis), perimortem	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	4	0	0	0	4
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0
Tubular protein, increased	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Infiltrate, interstitial, lymphoplasmacytic	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5. **MIDDLE KIDNE**Y, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail, karryorhexis)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	4	1	0	0	4
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	1	0	0	0
Skeletal muscle: Myositis, lymphohistiocytic and heterophilic, focal, mild, with myodegen and nec.	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Tubular protein, increased	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Infiltrate, interstitial, heterophilic	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, interstitial, lymphoplasmacytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6. CAUDAL KIDNEY, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail with karryorhexis)	0	0	0	0	0	0	0	0	0	0	1	0	2	3	0	0	1	1	4	0	0	0	4
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	1	1	0	0	0	0	2	1	0	0	0	0	0	0	0	0
Infiltrate, interstitial, lymphoplasmacytic	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7. Heart	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Myocarditis, subacute	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pigment, myocardial (mineral)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pericaridal fat, infiltrate, histiocytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8. Spleen and Bursa	P	P	P	P	P*	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Bursa, follicular cysts	1	2	0	0	NP	0	2	1	2	3	1	0	0	0	0	0	0	0	1	1	0	0	0
Bursa, lymphocytolysis or increased tingible body macrophages	0	0	0	0	NP	0	0	1	0	0	0	0	0	1	0	0	2	1	0	3	0	1	0
9. Thyroid gland (occ with thymus)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	0	0	0	0	0					2	0	0	0	0	0	0	0
10. Infundibulum, Magnum	P	P	P	P	P	P	P	P	P	P	P	NP	NP	NP	NP	NP	NP	P	NP	P	NP	NP	NP
Infiltrate, lymphoplasmacytic	0	0	0	0	0	1	0	0	0	0	0	.	.	.	.	.	.	.	.	0	.	.	.
11. Ovary, left	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Immature	0	0	0	0	0	0	0	0	0	0	0	3	4	4	3	3	3	0	3	0	2	0	3
12. Isthmus	P	P	P	P	NP	P	P	P	P	P	P	NP	NP	NP	NP	NP	NP	P	NP	P	NP	NP	NP
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	1	0	0	0	0	.	.	.	.	.	.		.	0	.	.	.
13. Shell gland (Uterus)/vagina	P	P	P	P	P	P	P	P	P	P	P	NP	NP	NP	NP	NP	NP	P	NP	P	NP	NP	NP

FEMALE Japanese Quail ID's are prefaced with '15-'	387	388	391	392	393	394	395	396	398	399	402	421	422	423	425	426	427	428	429	430	432	433	434
Female Dosage in mg/kg NTO-->	0	0	0	0	0	0	0	0	0	0	0	100	100	100	100	100	100	100	100	100	100	100	100
Date of euthanasia-->	5/20	5/20	5/20	5/20	5/20	5/20	5/20	5/21	5/20	5/20	5/20	5/19	5/19	5/19	5/19	5/20	5/20	5/20	5/20	5/21	5/21	5/21	5/21
Age at death (days)-->	86	86	86	86	86	86	86	87	86	86	86	85	85	85	85	86	86	86	86	87	87	87	87
1. Brain - cerebrum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2. Brain -cerebellum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
3. Liver	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Congestion	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, lymphoplasmacytic	1	0	0	1	1	0	0	0	0	0	0	3	0	0	0	1	0	0	1	0	0	0	0
Infiltrate, portal, heterophilic	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Hyperplasia, oval cell, portal, bridging	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Vacuolation, diffuse, random	1	0	2	1	0	1	3	0	1	4	2	3	1	0	0	0	0	0	0	0	3	1	0
Vacuolation, centrilobular	0	2	0	0	1	0	1	1	2	4	1	0	0	0	2	0	0	0	0	0	0	0	0
4. **CRANIAL KIDNEY**, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail, karryorhexis), perimortem	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tubular protein, increased	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, interstitial, lymphoplasmacytic	0	1	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	1	0	0	0	0
5. **MIDDLE KIDNE**Y, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail, karryorhexis)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Skeletal muscle: Myositis, lymphohistiocytic and heterophilic, focal, mild, with myodegen and nec.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tubular protein, increased	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, interstitial, heterophilic	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, interstitial, lymphoplasmacytic	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
6. CAUDAL KIDNEY, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail with karryorhexis)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Infiltrate, interstitial, lymphoplasmacytic	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1
7. Heart	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Myocarditis, subacute	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pigment, myocardial (mineral)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pericaridal fat, infiltrate, histiocytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8. Spleen and Bursa	P	P	P	P	P*	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Bursa, follicular cysts	1	2	0	0	NP	0	2	1	2	3	1	1	0	0	0	0	0	0	0	1	0	1	0
Bursa, lymphocytolysis or increased tingible body macrophages	0	0	0	0	NP	0	0	1	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0
9. Thyroid gland (occ with thymus)	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	NP	P	P	P	P	NP
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	.	0	.	0	0	0	1	.
10. Infundibulum, Magnum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Infiltrate, lymphoplasmacytic	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0
11. Ovary, left	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Immature	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12. Isthmus	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
13. Shell gland (Uterus)/vagina	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NP	P	P	P	P	P	P	P	P

First Filial Generation (F1) Females

FEMALE Japanese Quail ID's are prefaced with '15-'	681	682	683	684	685	686	687	689	690	691	692	693	695	714	715	716	717	719	720	721	722	723	724	725	726	727	728	729
Female Dosage in mg/kg NTO-->	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Date of euthanasia-->																												
Age at death (days)-->	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
1. Brain - cerebrum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2. Brain -cerebellum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
3. Liver	P	P	P	P	P	P	P	P	P	P	P	P	P	p	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Congestion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Infiltrate, lymphoplasmacytic	1	1	0	1	1	0	0	1	0	0	1	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	2	1
Infiltrate, portal, heterophilic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Hyperplasia, oval cell, portal, bridging	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	0	0	0	0	2	0
Vacuolation, diffuse, random	2	1	0	0	0	1	1	0	1	0	1	0	2	0	0	0	3	0	0	0	0	2	1	1	1	0	2	1
Vacuolation, centrilobular	1	1	1	1	3	0	0	3	0	4	0	1	0	2	0	3	0	3	1	1	2	0	0	0	0	0	1	3
4. **CRANIAL KIDNEY**, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail, karryorhexis), perimortem	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal tubules, single cell necrosis (perimortem)	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Tubular protein, increased	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, interstitial, lymphoplasmacytic	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
5. **MIDDLE KIDNEY**, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	0
Proximal Renal tubule Degeneration (loss of cytoplasmic detail, karryorhexis)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skeletal muscle: Myositis, lymphohistiocytic and heterophilic, focal, mild, with myodegen and nec.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tubular protein, increased	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, interstitial, heterophilic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, interstitial, lymphoplasmacytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
6. CAUDAL KIDNEY, Spinal cord, skeletal muscle, bone with marrow	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Proximal Renal tubule Degeneration (loss of cytoplasmic detail with karryorhexis)	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal tubules, single cell necrosis (perimortem)	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Infiltrate, interstitial, lymphoplasmacytic	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
7. Heart	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Myocarditis, subacute	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pigment, myocardial (mineral)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pericaridal fat, infiltrate, histiocytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8. Spleen and Bursa	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Bursa, follicular cysts	1	0	1	2	0	1	0	0	1	3	0	0	0	0	1	1	0	1	1	NP	0	NP	0	2	1	0	1	3
Bursa, lymphocytolysis or increased tingible body macrophages	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	.	0	.	0	1	0	0	0	0
9. Thyroid gland (occ with thymus)	P	P	P	P	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Infiltrate, lymphoplasmacytic	0	0	1	0	0	.	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0
10. Infundibulum, Magnum	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Infiltrate, lymphoplasmacytic	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
11. Ovary, left	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Immature	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12. Isthmus	P	P	P	P	P	P	P	P	P	P	NP	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Infiltrate, lymphoplasmacytic	0	1	0	0	0	0	0	0	0	0	.	.	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0
13. Shell gland (Uterus)/vagina	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

STORAGE OF STUDY MATERIALS AND RECORDS RETENTION

The study records and pathology final report will be archived and maintained at or under the direction of U.S. Army Public Health Center's Toxicology Directorate (TOX), according to TOX Standard Operating Procedures (SOP) and EPA requirements. The Pathology specimens will also be archived and maintained at or under the direction of USAPHC Toxicology Directorate, according to TOX SOP and EPA requirements.

FINAL REPORT APPROVAL

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22 September 2016

Erica E. Carroll, DVM, PhD, Diplomate ACVP
LTC, VC
Study Pathologist
Toxicology Directorate
U.S. Army Public Health Center

Date

Appendix O

Pathology Report B

ADDENDUM

PATHOLOGY REPORT

For

One-Generation Reproductive Toxicity Test in Japanese quail (Coturnix japonica) using 3-nitro-1,2,4-triazol-5-one (NTO)

Protocol No.: 80-14-07-02

Study Director:
Allison Jackovitz

Prepared by:

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Date: 2017.04.12 10:12:57 -04'00'

Keith Koistinen, DVM, Diplomate ACVP
Major, Veterinary Corps
Toxicology Directorate
U.S. Army Public Health Center

Date

Table of Contents

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This pathology investigation was conducted in a manner consistent with the principles of the United States Environmental Protection Agency (USEPA) Good Laboratory Practice regulations of the Toxic Substances Control Act (TSCA), as detailed in 40 CFR Part 792, plus amendments.

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Date

QUALITY ASSURANCE STATEMENT

For The Addendum to the Pathology Report for Protocol No. 80-14-07-02 entitled "One-Generation Reproductive Toxicity Test in Japanese quail (Coturnix japonica) using 3-nitro-1,2,4-triazol-5-one (NTO)", the following critical phases were audited by the APHC Quality Systems and Regulatory Compliance Office (QSARC), Laboratory and Toxicology Accreditation and Compliance Office (LTACO):

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Pathology Contributing Scientist Inspection - Summary Data and Summary Table Review	03/18/2017	04/11/2017
Pathology Contributing Scientist Inspection- Final Pathology Report GLP Standard Regulation Review	03/18/2017	04/11/2017

Note 1 All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Note 2 In addition to the study specific critical phase inspections listed here, general facility and process based inspections not specifically related to this study are done monthly or annually in accordance with QSARC, LTACO Standing Operating Procedures.

Note 3 This report has been audited by the Quality Assurance Unit (QSARC, LTACO) and is considered to be an accurate account of the data generated and of the procedures followed

Michael P. Kefauver

Michael P. Kefauver
Quality Assurance Specialist, QSARC

04/11/2017

Date

BACKGROUND

The study director requested a secondary review of the brainstem and cerebellum sections because vacuolar changes were observed in a dose-dependent manner, i.e. vacuoles were present in the high dose groups but were absent in the control groups, and these vacuoles correlated to the observed clinical signs. In the original report the vacuoles were interpreted to be an insignificant artifactual change that developed due to exposure of the tissue to alcohol or post-mortem autolysis.

The primary objective of this extended-one-generation study was to determine whether 3-nitro-1,2,4-triazol-5-one (NTO) is an endocrine disrupting chemical (EDC) and secondarily to determine the immunological effects of NTO. As such, this study was not optimized to assess NTO's neurotoxicity and therefore the tissue processing steps were not optimized to assess these effects. These acute effects were not anticipated because NTO is not acutely toxic in rodents and the LD50 in this species is greater than 5000 mg/kg.

METHODS

The sections of cerebellum and brainstem from animals in all study groups were analyzed by the reviewing pathologist. The cerebellum and brainstem of the mid-low (100 mg/kg/day) and the low-dose group (20 mg/kg/day) were processed and analyzed for this report; the brains from the birds in these groups were collected and fixed in formalin at necropsy, but no additional processing or analysis was performed on these tissues for the original pathology report. These tissues were not processed because no toxic effect was noted in the brain, therefore only the two highest dose groups (500 and 1000 mg/kg/day) were processed and analyzed for the original report.

As an adjunct to the routine H&E stained sections, sections of the brain from four birds were analyzed immunohistochemically with glial fibrillary acidic protein (GFAP) in order to characterize the vacuolar change; GFAP is a marker of mature astrocytes, however it is not entirely specific for astrocytes.

RESULTS

Clinical Signs and mortality:

All (32 of 32) of the birds in the high-dose (1000 mg/kg/day) and 97% (30 of 31) of the birds in the mid-high dose (500 mg/kg/day) were found dead or were sacrificed early due to loss of 20% of the body mass. Neurologic deficits, ataxia and other neuromuscular deficiencies including convulsions were observed in all of these birds. The birds alternated between prostrate inactivity and ataxia beginning 3-4 hours after treatment and these clinical signs went away approximately 12 hours after treatment, which suggests that that the compound had been excreted or metabolized. With repeated daily exposure, periods of convulsions, ataxia, and inactivity lengthened to where the birds were still ataxic 24 hours after dosing. Loss of body mass was primarily attributed to inability to stand and eat. The period between commencement of exposure to NTO and appearance of neurologic deficiencies was shorter in the high dose group (1000 mg/kg/day) compared to the mid-high dose group (500 mg/kg/day); neurological deficits were observed following 5 and 17 days of treatment, in each group, respectively. Similar to commencement of clinical signs

the animals in the high-dose group died earlier than those in other groups, among the birds that died prior to the end of the study, animals in 1000 mg/kg and 500 mg/kg groups, were between 29-40 or 32-79 days of age at death, respectively. See table below for the full range of age at deaths for males and females for all 62 birds that were had premature deaths (excluding the one bird in the 500 mg/kg/day group that survived until the end of the study).

Table 1: Days of age at death, for deaths prior to the end of the study.

		1000 mg/kg	500 mg/kg
Males and Females	Minimum	29	32
	Maximum	40	79
	Average	40.0	44.6
Males	Minimum	30	32
	Maximum	39	79
	Average	34.4	46.1
Females	Minimum	29	33
	Maximum	40	56
	Average	34.8	42.3

Histopathology:

Vacuolization of the cerebellum and/or the brainstem were observed on histopathologic examination, and these changes were present in a dose dependent manner. Vacuoles within the cerebellum were observed in all sections examined for the birds in the two highest dose groups. The cerebellum was not adequate for examination for several birds (12 of 22 birds in 1000 mg/kg/day, 8 of 31 birds in 500 mg/kg/day) due to processing related loss or it was not present in the plane of section. Three of the high-dose group birds (1000 mg/kg/day) had brainstem but not cerebellum available for examination. Within 4 of 12 males and 3 of 12 females in the mid-low dose group (100 mg/kg/day) vacuoles were noted in brainstem nuclei, but not the deep cerebellar nuclei.

Neuropil vacuoles were not noted in any animal in the low dose group (20 mg/kg /day) or the control group. Four birds, one male control (0 mg/kg) bird (15-293), and three from the lowest dose group (20 mg/kg; 15-323, 15-405, and 15-410), were sacrificed/died prior to the end of the study, and vacuoles were not observed in these birds. These birds were euthanized on days, 31, 32, 56, and 46 days of age, respectively. Additionally, one male bird in the mid-high (500 mg/kg/day) dose group survived until the end of the study (15-348) and vacuoles were present in the cerebellum and brainstem, and these vacuoles were similar distribution and severity to the birds that were sacrificed/died prematurely.

The vacuoles within the grey matter neuropil are 25-40 microns in diameter, are empty or contain a small amount of eosinophilic material, have regularly round smooth edges. The edges of the vacuoles are

highlighted with the GFAP (Figure 2), and are frequently located immediately adjacent to the neuronal cell body or capillaries.

One female bird in the 20 mg/kg dose group had a focal area with cerebellar heterotopia or dysplasia, characterized by inversion of the normal cerebellar layers with normal appearing neurons in the incorrect location. This is likely an incidental finding that is unrelated to the study treatment or design.

Table 2

Sex-->	M	F	M	F	M	F	M	F	M	F
Dosage in mg/kg NTO-->	0	0	20	20	100	100	500	500	1000	1000
Cerebellum vacuoles-deep cerebellar white or gray matter							14/14	9/9	14/14	7/7
Brainstem - vacuoles	0/11	0/11	0/13	0/12	4/12	3/12	12/14	9/9	14/15	8/9
Cerebellum or brainstem - vacuoles					4/12	3/12	14/14	9/9	14/15	8/9

Blank cell indicates absence of observation in that group

Sex-->	M	F	M	F	M	F	M	F	M	F
Dosage in mg/kg NTO-->	0	0	20	20	100	100	500	500	1000	1000
Cerebellum vacuoles-deep cerebellar white or gray matter							100%	100%	100%	100%
Brainstem - vacuoles	0	0	0	0	33%	25%	86%	100%	93%	89%
Cerebellum or brainstem - vacuoles					33%	25%	100%	100%	93%	89%

Blank cell indicates absence of observation in that group

DISCUSSION

Based on distribution among groups and consistent anatomic distribution among effected birds, the vacuoles within the brainstem and cerebellum in this study likely formed due to exposure to the test article, but formation of the vacuoles may have occurred during the post-mortem period. The most likely explanation for formation of the vacuoles is that they formed secondary to stimulation of the astrocytic cell metabolism or pertubation of the aquaporin channels, which subsequently resulted in postmortem swelling of the astrocyte cell processes. Thus, in fact, this altered astrocytic morphology may represent an artifact (albeit a helpful and consistent one) of immersion fixation.

From a functional neurology aspect, the location of vacuoles within the cerebellum correlates well with the neurologic signs that were observed in the two highest dose groups. The anatomic location of the lesion was consistently present within the deep cerebellar nucleus and within the adjacent peduncles of the brainstem among the high dose groups. The consistency between animals suggests that the vacuolization is distributed in a bilaterally symmetrically manner; note, determining whether the change was bilaterally symmetrical is not definitively possible because the sections examined were prepared in a sagittal plane. Within the mid-dose group (100 mg/kg/day) the change was less frequent and was found only within the brainstem nuclei.

One of the many known roles of the astrocyte is to remove and detoxify ammonia, since the test article is a nitrogenous chemical, metabolism of the toxicant or one its metabolites by astrocytes should be investigated.

The conclusion that this change developed in the post-mortem period is supported by other reports that have characterized similar astrocyte swelling/vacuolation as a post-mortem change, by comparing cryostat sections from snap-frozen brains, which did not have vacuoles, with paraffin sections from similarly treated animals, which had prominent vacuoles.[1] A comparison such as this is required to further characterize whether this change is an ante- or a post-mortem change.

In cases of hyperammonemia, due to causes such as hepatic encephalopathy, there is well characterized swelling of astrocyte nuclei known as formation of Type II astrocytes. Ammonia is efficiently converted into glutamine within the cytoplasm of astrocytes; this action protects adjacent neurons from toxicity at the expense of poisoning the astrocytes.[2-4] A potential mechanism by which there is glutamine-induced astrocytic swelling includes osmosis of the extracellular water down a steep solute gradient resulting in intracellular swelling.[2] In cases of hyperammonemia the altered astrocytic morphology (Type II astrocytes) is not apparent in perfusion-fixed tissues,[5] indicating that this hallmark brain lesion of altered astrocytic morphology may represent an artifact of immersion fixation; immersion fixation is suspected to play a role for development of this vacuole development for animals in this study.

The specific cellular location of the vacuoles cannot be determined at the light microscopic level, but the tendency of the vacuoles to be adjacent to neurons and vessels suggests that the vacuoles are within astrocytic cell processes. The thin rim of GFAP stained material around the vacuoles also supports the interpretation that the observed vacuoles represent swelling of astrocytes. Additional analysis with electron microscopy and/or immunoelectron microscopy analysis is required to provide cellular location with certainty.

Similar vacuoles have been reported as a post-mortem change that is enhanced by pharmaceutical treatments; these reports speculate that vacuole formation appears to be secondary to altered level of astrocyte activity caused by the test article.[1] Astrocytic swelling/vacuolation has also been attributed to several toxicants including 6-aminonicotinamide (6-AN);[6,7] chlorosugars,[8] dinitrobenzene, and tribromoimidazole,[9] The mechanism by which these toxicants cause astrocytic swelling is thought to be due to acute energy deprivation resulting from impaired glucose utilization via the glycolytic pathway.[7,9,10] The vacuolation of astrocytes has also been observed following Ampakine exposure (Garman personal communication); this class of pharmaceutical drugs strongly interacts with the glutamatergic AMPA receptor.

The reasons for development of the observations provided in the original report, i.e., immersion of tissues in alcohol fixative and post-mortem autolysis are not appropriate. Alcohol was not used as a fixative and prolonged tissue alcohol exposure did not occur. Post-mortem autolysis is not an appropriate reason for development of the vacuoles due to the following reasons: 1) most (75%) of the birds were necropsied immediately after euthanasia, 2) there is no correlation between post-mortem tissue fixation interval and incidence of vacuolation i.e., vacuoles are not more severe or more prevalent in animals with a longer death to tissue fixation interval, and 3) there is good cellular preservation in tissue sections that have vacuoles without other evidence of significant post-mortem autolysis.

To the extent that comparison could be made, the age at time of death had no effect on the formation of vacuoles. Exact one-to-one comparison of age-matched controls for the animals with early deaths is not possible since all but one of the birds in the two highest dose groups died prior to the end of the study and only four animals from the lowest two dose groups (one control and three low-dose) died prematurely. Among these four animals the vacuoles were not present, which supports the conclusion that the cerebellar vacuolation was not merely an effect of age. Additionally, one male bird from the 500 mg/kg treatment group survived until the end of the study and had vacuoles that were similar in appearance as birds with premature death. Furthermore, there are no known changes in the brain from 35 days of age to 85 that would account for astrocyte swelling/vacuolation observed in this study.

Other nitroaromatic compounds including hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 1, 3, 5-trinitrobenzene (TNB) cause CNS dysfunction. RDX specifically causes grand mal seizures, after oral exposure, which is thought to be mediated through inhibition of the GABA receptor, but neuorohistopathologic lesions were not apparent after 13 weeks of oral exposure in rats[11] and after a 14 day oral exposure in northern bobwhite quail.[12]

CONCLUSION

The vacuoles in these birds are most likely present within astrocyte processes and cytoplasm. The exact mechanism for which the vacuoles in these birds developed is unknown, but the development of the vacuoles is most likely secondary to exposure of the test article. Similar to the effects of ammonia on astrocytes from hyperammonemia, the test article may have caused increased activation or metabolism process by astrocytes resulting in the development of the vacuoles. Other nitroaromatic compounds including RDX and TNB cause neurologic deficits that is thought to be mediated through the GABA receptor, but a direct histopathologic comparison cannot be made to these toxicants because histopathologic lesions were not apparent in studies that have examined the CNS tissues following exposure to these compounds. Additional investigation is warranted to elucidate the mechanism by which NTO induces the neurologic deficits and vacuoles within the cerebellum and brainstem observed in these birds.

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PHOTOMICROGRAPHS

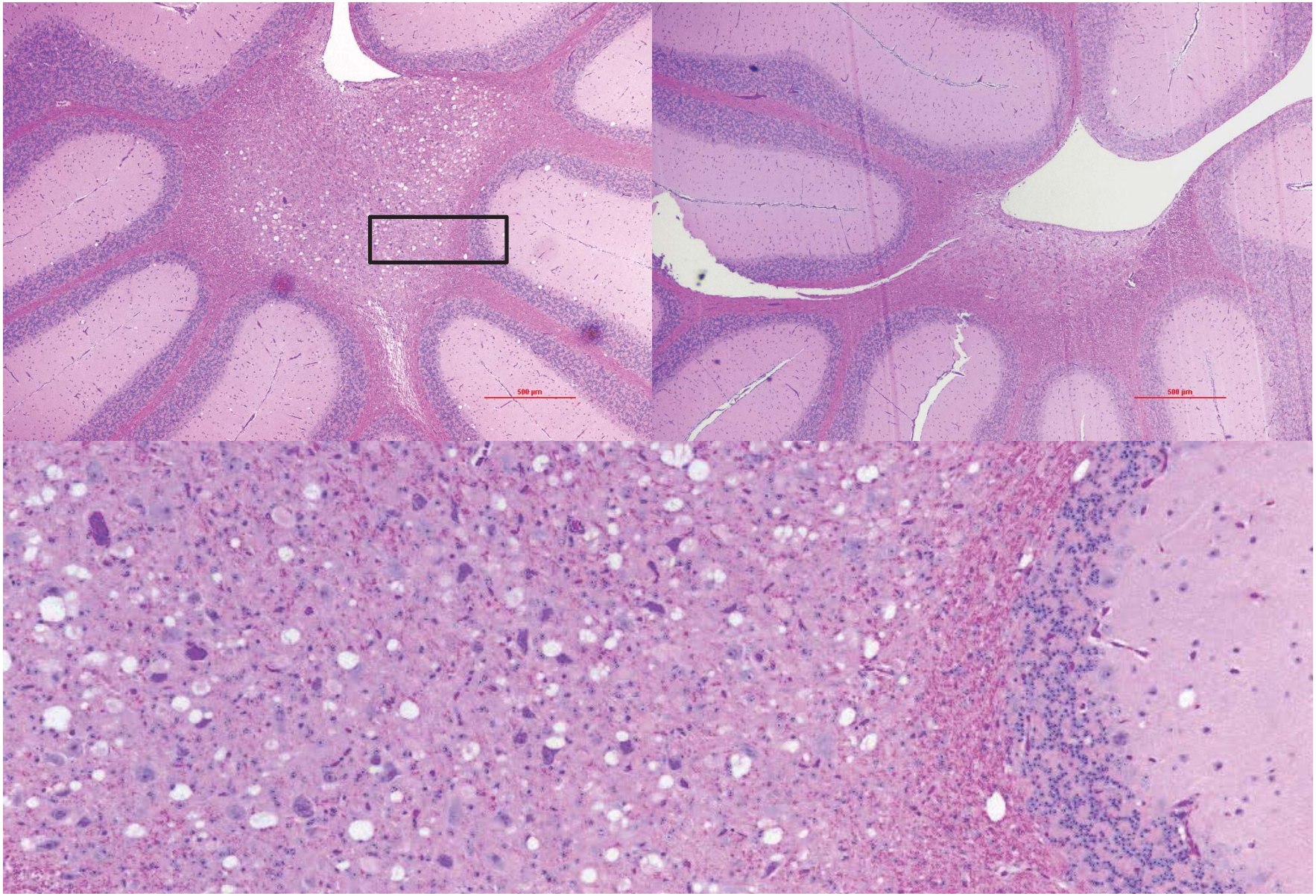

Figure 1: Within the deep cerebellar nucleus of the high dose male bird (1000 mg/kg/day, #15-368) top left, there are numerous vacuoles that are absent in the control animal (#15-293), top right.. Lower panel, higher magnification of the area outlined by the box in the top right photo. The vacuoles are empty and clear or contain a small amount ot eosinophilic material. These birds died at similar days of age, day 39 and day 31; #15-368 and #15-293, respectively.

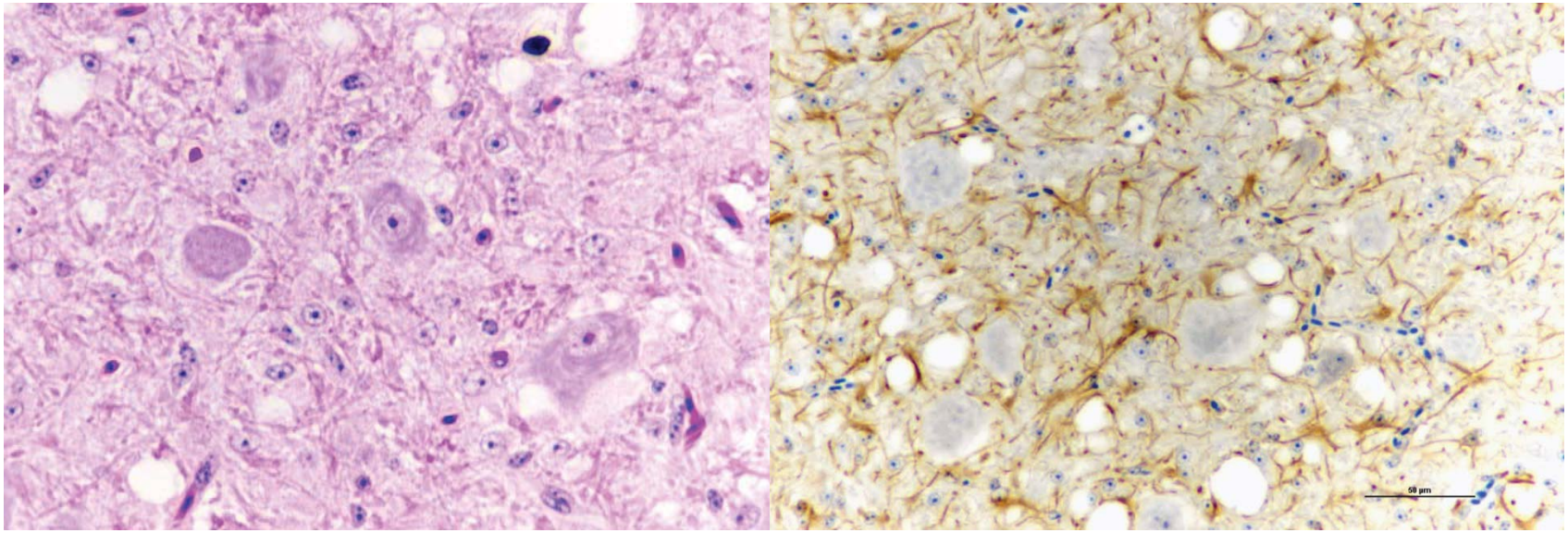

Figure 2: Cerebellum, deep cerebellar nucleus, high dose male (1000 mg/kg/day), #15-358, H&E stain left and glial fibrillary acidic protein (GFAP) immunohistochemical (IHC) stain right. The vacuoles within the grey matter neuropil are 25-40 microns in diameter, are empty or contain a small amount of eosinophilic material, have regularly round smooth edges that are frequently highlighted with GFAP (right panel), and are frequently located immediately adjacent to the neuronal cell body or capillaries.

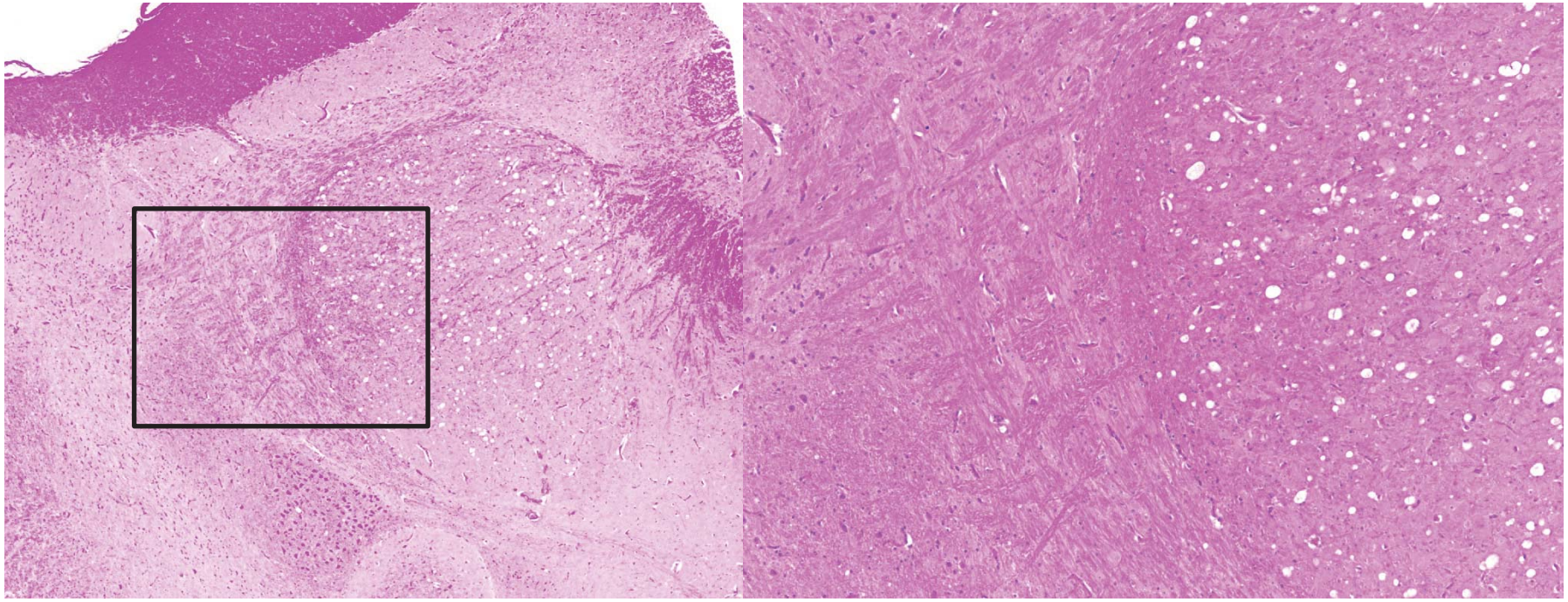

Figure 3: Brainstem, mid-low dose male (100 mg/kg/day), #15-340, right panel, higher magnification of area outlined by the box in the left panel. Astrocytes within a brainstem nucleus contain numerous vacuoles, while the adjacent brainstem parenchyma and cerebellum is unaffected (cerebellum not shown).

HISTOPATHOLOGY FINDINGS, BY ANIMAL

Table 3: 0 mg/kg/day group Males and Females

All Identification numbers are prefaced with '15-'	284	285	286	287	288	289	291	292	293	294	296	297	387	388	391	392	393	394	395	396	398	399	402		
Generation-->	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0		
Date of euthanasia-->	5/19	5/19	5/19	5/19	5/20	5/20	5/20	5/21	3/26	5/21	5/21	5/21	5/19	5/19	5/19	5/20	5/20	5/20	5/20	5/21	5/21	5/21	5/21		
Date of Necropsy	5/19	5/19	5/19	5/19	5/20	5/20	5/20	5/21	3/26	5/21	5/21	5/21	5/19	5/19	5/19	5/20	5/20	5/20	5/20	5/21	5/21	5/21	5/21		
Death to necropsy interval (hrs)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
AGE in days at death-->	86	86	86	86	86	86	86	86	31	86	86	86	86	86	86	86	86	86	86	87	86	86	86	Lesion Incidence by group	
Sex-->	M	M	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F	F	M	F
Tissue Dosage in mg/kg NTO-->	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cerebellum - Present	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	11	11
Cerebellum - Absent or not enough sample available for evaluation						1																		1	0
Cerebellum - Essentially normal tissue	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	11	11
Cerebellum - Neuropil vacuoles-deep cerebellar white or gray																								0	0
Cerebellum- Heterotopia, dysplasia of cerebellar layer inversion																								0	0
Brainstem - Present	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	11	11
Brainstem - Absent or not enough sample available for evaluation						1																		1	0
Brainstem - Essentially normal tissue	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	11	11
Brainstem- Neuropil vacuoles																								0	0
Cerebellum or brainstem present	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	11	11
Cerebellum and brainstem - Absent						1																		1	0
Cerebellum and brainstem - Essentially normal tissue	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	11	11
Cerebellum and/or brainstem - Vacuolation																								0	0
																							# of animals in group	12	11

Scoring criteria: 0 ='zero to <1 % of tissue is affected; 1 = < 5% of tissue is affected (minimal); 2= 6-15% of tissue is affected (mild); 3= 16-40% of tissue is affected (moderate); 4= >41% of tissue is affected (marked).

Table 3 (continued): 100 mg/kg/day group Males and Females

All Identification numbers are prefaced with '15-'	312	314	315	316	317	318	319	320	321	322	323	324	327		403	404	405	406	407	408	409	410	412	413	414	415	
Generation-->	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0		F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	
Date of euthanasia-->	5/19	5/19	5/19	5/19	5/20	5/20	5/20	5/20	5/20	5/21	3/27	5/21	5/21		5/19	5/19	4/20	5/19	5/19	5/20	5/20	4/10	5/20	5/21	5/21	5/21	
Date of Necropsy	5/19	5/19	5/19	5/19	5/20	5/20	5/20	5/20	5/20	5/21	3/27	5/21	5/21		5/19	5/19	4/20	5/19	5/19	5/20	5/20	4/10	5/20	5/21	5/21	5/21	
Death to necropsy interval (hrs)	0	0	0	0	0	0	0	0	0	0	1	0	0		0	0	<24 hrs	FD ?	0	0	0	FD	0	0	0	0	
AGE in days at death-->	85	85	85	85	86	86	86	86	86	87	32	87	87	Totals	85	85	56	85	85	86	86	46	86	87	87	87	Totals
Sex-->	M	M	M	M	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F	F	F	F
Tissue Dosage in mg/kg NTO-->	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Cerebellum - Present	1	1		1	1	1	1	1	1	1	1	1	1	12	1	1	1	1	1	1	1	1	1		1	1	11
Cerebellum - Absent or not enough sample available for evaluation			1											1										1			1
Cerebellum - Essentially normal tissue	1	1		1	1	1	1	1	1	1	1	1	1	12	1	1	1	1		1	1	1	1		1	1	10
Cerebellum - Neuropil vacuoles-deep cerebellar white or gray														0													0
Cerebellum- Heterotopia, dysplasia of cerebellar layer inversion														0					1								1
Brainstem - Present	1	1	1	1	1	1	1	1	1	1	1	1	1	13	1	1	1	1	1	1	1	1	1	1	1	1	12
Brainstem - Absent or not enough sample available for evaluation														0													0
Brainstem - Essentially normal tissue	1	1	1	1	1	1	1	1	1	1	1	1	1	13	1	1	1	1	1	1	1	1	1	1	1	1	12
Brainstem- Neuropil vacuoles														0													0
Cerebellum or brainstem present	1	1	1	1	1	1	1	1	1	1	1	1	1	13	1	1	1	1	1	1	1	1	1	1	1	1	12
Cerebellum and brainstem - Absent														0													0
Cerebellum and brainstem - Essentially normal tissue	1	1	1	1	1	1	1	1	1	1	1	1	1	13	1	1	1	1	1	1	1	1	1	1	1	1	12
Cerebellum and/or brainstem - Vacuolation														0													0
														13												# of animals in group	12

Scoring criteria: 0 ='zero to <1 % of tissue is affected; 1 = < 5% of tissue is affected (minimal); 2= 6-15% of tissue is affected (mild); 3= 16-40% of tissue is affected (moderate); 4= >41% of tissue is affected (marked).

Table 3 (continued): 100 mg/kg/day group Males and Females

ID # 15-XXX-->	329	330	332	333	334	335	337	338	339	340	341	343		421	422	423	425	426	427	428	429	430	432	433	434	
Generation-->	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0		F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	
Date of euthanasia-->	5/19	5/19	5/19	5/20	5/20	5/20	5/20	5/21	5/1	5/21	5/21	5/21		5/19	5/19	5/19	3/31	5/20	5/20	5/20	5/20	5/21	5/21	5/21	5/21	
Date of Necropsy	5/19	5/19	5/19	5/20	5/20	5/20	5/20	5/21	5/1	5/21	5/21	5/21		5/19	5/19	5/19	3/31	5/20	5/20	5/20	5/20	5/21	5/21	5/21	5/21	
Death to necropsy interval (hrs)	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	
AGE in days at death-->	85	85	85	86	86	86	86	87	67	87	87	87	Totals	85	85	85	85	86	86	86	86	87	87	87	87	Totals
Sex-->	M	M	M	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F	F	F	F
Tissue Dosage ir	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Cerebellum - Present	1	1	1	1	1	1	1	1	1	1	1	1	12			1		1	1	1	1	1	1	1	1	9
Cerebellum - Absent or not enough sample available for evaluation													0	1	1		1									3
Cerebellum - Essentially normal tissue	1	1	1	1	1	1	1	1	1	1	1	1	12			1		1	1	1	1	1	1	1	1	9
Cerebellum - Neuropil vacuoles-deep cerebellar white or gray													0													0
Cerebellum- Dysplasia, inversion of layers													0													0
Brainstem - Present	1	1	1	1	1	1	1	1	1	1	1	1	12	1	1	1	1	1	1	1	1	1	1	1	1	12
Brainstem - Absent or not enough sample available for evaluation													0													0
Brainstem - Essentially normal tissue	1	1	1			1		1	1		1	1	8			1	1		1	1	1	1	1	1	1	9
Brainstem- Neuropil vacuoles				1	1		1			1			4	1	1			1								3
Cerebellum or brainstem present	1	1	1	1	1	1	1	1	1	1	1	1	12	1	1	1	1	1	1	1	1	1	1	1	1	12
Cerebellum and brainstem - Absent													0													0
Cerebellum and brainstem - Essentially no	1	1	1			1		1	1		1	1	8			1	1		1	1	1	1	1	1	1	9
Cerebellum and/or brainstem - Vacuolation				1	1		1			1			4	1	1			1								3
												# of animals in group	12												# of animals in group	12

Scoring criteria: 0 ='zero to <1 % of tissue is affected; 1 = < 5% of tissue is affected (minimal); 2= 6-15% of tissue is affected (mild); 3= 16-40% of tissue is affected (moderate); 4= >41% of tissue is affected (marked).

Table 3 (continued): 500 mg/kg/day group Males and Females

ID # 15-XXX -->	344	346	347	348	350	351	352	353	354	355	356	357	358	359	360	362	363	364	365		435	438	439	440	441	442	443	445	446	447	448	450	
Generation-->	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0		F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	
Date of euthanasia-->	4/27	4/10	4/6	5/19	4/6	4/20	4/6	4/10	4/6	4/10	4/2	4/6	4/3	4/10	4/8	5/12	4/1	3/27	3/26		4/6	3/30	3/29	4/6	4/9	4/8	4/20	3/28	4/20	4/9	4/6	3/28	
Date of Necropsy	4/27	4/10	4/6	5/19	4/6	4/20	4/6	4/10	4/6	4/10	4/2	4/6	4/3	4/10	4/8	5/12	4/1	3/27	3/26		4/6	3/30	3/30	4/6	4/9	4/8	4/20	3/30	4/20	4/9	4/6	3/30	
Death to necropsy interval (hrs)	0	0	0	0	FD	0	0	0	0	?	0	0	0	Unknov	0	0	1	2	<24 hrs		0	0	17	0	0	?	<24 h	52	<24 h	0	?	52	
AGE in days at death-->	64	47	43	86	43	57	43	47	43	47	39	43	40	47	45	79	38	33	32	Total	42	35	34	42	45	44	56	33	56	45	42	33	Total
Sex-->	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F	F	F	F
Tissue Dosa	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500
Cerebellum - Present		1	1	1			1	1		1	1	1	1	1	1	1	1	1		14	1	1	1		1	1		1		1	1	1	9
Cerebellum - Absent or not enough sample	1				1	1			1										1	5				1			1		1				3
- Essentially normal tissue																				0													0
- Neuropil vacuoles-deep cerebellar white		1	1	1			1	1		1	1	1	1	1	1	1	1	1		14	1	1	1		1	1		1		2	1	2	9
- Dysplasia, inversion of layers																				0													0
Brainstem - Present		1	1	1			1	1		1	1	1	1	1	1	1	1	1		14	1	1	1		1	1		1		1	1	1	9
- Absent or not enough sample available fo	1				1	1			1										1	5				1			1		1				3
- Essentially normal tissue												1	1							2													0
- Neuropil vacuoles		1	1	1			1	1		2	1			1	1	1	1	1		12	1	1	1			1		1		1	1	1	8
Cerebellum or brainstem present		1	1	1			1	1		1	1	1	1	1	1	1	1	1		14	1	1	1		1	1		1		1	1	1	9
Cerebellum and brainstem - Absent	1				1	1			1										1	5				1			1		1				3
Cerebellum and brainstem - Essentially normal tissue																				0													0
Cerebellum and/or brainstem - Vacuolation		1	1	1			1	1		1	1	1	1	1	1	1	1	1		14	1	1	1		1	1		1		1	1	1	9
																		# of animals in group		19													12

Scoring criteria: 0 ='zero to <1 % of tissue is affected; 1 = < 5% of tissue is affected (minimal); 2= 6-15% of tissue is affected (mild); 3= 16-40% of tissue is affected (moderate); 4= >41% of tissue is affected (marked).

Table 3 (continued): 1000 mg/kg/day group Males and Females

ID # 15-XXX -->	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385		451	452	454	455	456	457	458	459	460	461	462	463	
Generation-->	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0		F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	F0	
Date of euthanasia-->	3/29	3/30	4/3	4/3	3/26	3/29	3/26	3/28	4/1	3/30	3/25	3/28	3/27	3/25	3/31	3/30	3/28	3/25	4/1	4/1		3/29	3/30	3/31	3/26	4/3	3/27	3/26	3/25	4/3	3/28	3/24	4/3	
Date of Necropsy-->	3/30	3/30	4/3	4/3	3/26	3/30	3/26	3/30	4/1	3/30	3/25	3/30	3/27	3/25	4/1	3/30	3/30	3/25	4/1	4/1		3/30	3/30	3/31	3/26	4/3	3/27	3/26	3/25	4/3	3/30	3/24	4/3	
Death to necropsy interval (hrs)	29	0	0	0	0	29	0	52	0	0	1	52	1	1	FD	0	52	0	0	0		26	0	0	0	0	0	0	1	0	52	FD	0	
AGE in days at death-->	34	35	39	39	31	34	31	33	37	35	30	33	32	30	36	35	33	37	37	37	Totals	35	36	37	29	40	33	32	31	40	34	31	40	Totals
Sex-->	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F	F	F	F
Tissue Dosage in mg	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Cerebellum - Present	1	1	1	1		1		1	1	1			1		1	1	1		1	1	14		1	1	1	1				1	1		1	7
Cerebellum - Absent or not enough sample available for evaluat					1		1				1	1		1				1			6	1					1	1	1			1		5
Cerebellum - Essentially normal tissue																					0													0
Cerebellum - Neuropil vacuoles-d	2	2	2	2		1		2	2	1			1		1	2	1		1	1	14		1	1	2	1				1	1		1	7
Cerebellum- Dysplasia, inversion of layers																					0													0
Brainstem - Present	1	1	1	1		1		1	1	1		1	1		1	1	1		1	1	15	1	1	1	1	1	1			1	1		1	9
Brainstem - Absent or not enough sample available for evaluatio					1		1				1			1				1			5							1	1			1		3
Brainstem - Essentially normal tissue												1									1	1												1
Brainstem- Neuropil vacuoles	1	2	1	1		1		1	2	1			1		1	1	1		1	1	14		1	1	1	1	1			1	1		1	8
Cerebellum or brainstem present	1	1	1	1		1		1	1	1		1	1		1	1	1		1	1	15	1	1	1	1	1	1			1	1		1	9
Cerebellum and brainstem - Absent					1		1				1			1				1			5							1	1			1		3
Cerebellum and brainstem - Essentially normal tissue												1									1	1												1
Cerebellum and/or brainstem - Va	1	1	1	1		1		1	1	1			1		1	1	1		1	1	14		1	1	1	1	1			1	1		1	8
																					# of animals in group	20											# of animals in group	12

Scoring criteria: 0 ='zero to <1 % of tissue is affected; 1 = < 5% of tissue is affected (minimal); 2= 6-15% of tissue is affected (mild); 3= 16-40% of tissue is affected (moderate); 4= >41% of tissue is affected (marked).

STATISTICAL ANALYSES

Statistical analysis - sexes combined

							Is there a difference?	Compare Control Group to Other Doses		Compare All Dose Groups to Each Other	
Type	Tissue	Dose	# of Quail	1	0	Proportion	Chi-squared p-value	Fisher's p-values	Conclusions	Fisher's p-values	Conclusions
Brainstem	Brainstem- Neuropil vacuoles	0	22	0	22	0/22					
		20	25	0	25	0/25		1.0000			D0 & D20 < D100 < D500 & D1000
		100	24	7	17	7/24	0.0000	0.0100	D0 < D100	0.1000	
		500	23	20	3	20/23		0.0000	D0 < D500	0.0000	
		1000	24	22	2	22/24		0.0000	D0 < D1000		
Cerebellum	Cerebellum - Neuropil vacuoles-deep cerebellar white or gray	0	22	0	22	0/22					
		20	25	0	25	0/25		1.0000			D0 & D20 & D100 < D500 & D1000
		100	24	0	24	0/24	0.0000	1.0000			
		500	23	23	0	23/23		0.0000	D0 < D500	0.0000	
		1000	24	21	3	21/24		0.0000	D0 < D1000		
Cerebellum or brainstem	Cerebellum or brainstem - Vacuolation	0	22	0	22	0/22					
		20	25	0	25	0/25		1.0000			D0 & D20 < D100 < D500 & D1000
		100	24	7	17	7/24	0.0000	0.0100	D0 < D100	0.0100	
		500	23	23	0	23/23		0.0000	D0 < D500	0.0000	
		1000	24	22	2	22/24		0.0000	D0 < D1000		

Dose 100 Neuropil Vacuoles: Brainstem 7/24 and Cerebellum 0/24 p = 0.009 Brainstem > Cerebellum

Statistical analysis – Separate sexes

								Is there a difference?	Compare Control Group to Other Doses		Compare All Dose Groups to Each Other	
Type	Tissue	Sex	Dose	# of Quail	1	0	Proportion	Chi-squared p-value	Fisher's p-values	Conclusions	Fisher's p-values	Conclusions
Brainstem	Brainstem- Neuropil vacuoles	F	0	11	0	11	0/11	0.0000				D0 & D20 & D100 < D500 & D1000
			20	12	0	12	0/12		1.0000			
			100	12	3	9	3/12		0.2170			
			500	9	8	1	8/9		0.0000	D0 < D500	0.0080	
			1000	9	8	1	8/9		0.0000	D0 < 1000		
		M	0	11	0	11	0/11	0.0000				D0 & D20 < D100 < D500 & D1000
			20	13	0	13	0/13		1.0000			
			100	12	4	8	4/12		0.0930		0.0390	
			500	14	12	2	12/14		0.0000	D0 < D500	0.0140	
			1000	15	14	1	14/15		0.0000	D0 < 1000		
Cerebellum	Cerebellum - Neuropil vacuoles-deep cerebellar white or gray	F	0	11	0	11	0/11	0.0000				D0 & D20 & D100 < D500 & D1000
			20	12	0	12	0/12		1.0000			
			100	12	0	12	0/12		1.0000			
			500	9	9	0	9/9		0.0000	D0 < D500	0.0000	
			1000	9	7	2	7/9		0.0000	D0 < 1000		
		M	0	11	0	11	0/11	0.0000				D0 & D20 & D100 < D500 & D1000
			20	13	0	13	0/13		1.0000			
			100	12	0	12	0/12		1.0000			
			500	14	14	0	14/14		0.0000	D0 < D500	0.0000	
			1000	15	14	1	14/15		0.0000	D0 < 1000		
Cerebellum or brainstem	Cerebellum or brainstem Vacuolation	F	0	11	0	11	0/11	0.0000				D0 & D20 & D100 < D500 & D1000
			20	12	0	12	0/12		1.0000			
			100	12	3	9	3/12		0.2170			
			500	9	9	0	9/9		0.0000	D0 < D500	0.0080	
			1000	9	8	1	8/9		0.0000	D0 < 1000		
		M	0	11	0	11	0/11	0.0000				D0 & D20 < D100 < D500 & D1000
			20	13	0	13	0/13		1.0000			
			100	12	4	8	4/12		0.0930		0.0390	
			500	14	14	0	14/14		0.0000	D0 < D500	0.0030	
			1000	15	14	1	14/15		0.0000	D0 < 1000		

There were no significant differences in the proportions between the genders.

STORAGE OF STUDY MATERIALS AND RECORDS RETENTION

The study records and pathology final report will be archived and maintained at or under the direction of U.S. Army Public Health Center's (APHC) Toxicology Portfolio (TOX), according to TOX SOPs and EPA requirements. The Pathology specimens will also be archived and maintained at or under the direction of APHC Toxicology Portfolio, according to TOX SOP and EPA requirements.

Appendix P

Individual and Summary Clinical Chemistry Data

Table P-1
12 Week Individual Clinical Chemistry Analyses
F0 Generation Female Quail

Dose Group	Animal ID	ALB (g/dL)	AST (U/L)	Ca (mg/dL)	GGT (U/L)	GLOB (mg/dL)	TP (mg/dL)	URIC (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
	Y1	1.2	150	27.6	7	3.2	4.4	4.9	149	2.7	118
	Y4	0.9	120	21.9	3	2.8	3.7	7.4	148	4.7	117
Control	Y11	0.6	149	24.8	4	3.2	3.8	8.6	144	3.7	116
	Y22	1.1	198	32.0	5	3.4	4.5	5.6	149	3.6	117
	Y23	1.1	239	32.6	4	3.6	4.6	5.7	146	3.9	113
	Y24	1.2	141	37.5	7	3.5	4.7	8.9	150	2.6	118
	Y29	1.1	197	33.8	7	3.6	4.7	8.6	148	5.1	114
	Y30	1.0	175	21.1	5	3.1	4.0	6.2	151	3.2	115
	Y34	0.8	122	23.8	8	3.1	3.9	5.9	147	4.3	117
	Y46	0.9	311	30.0	9	3.4	4.4	4.8	148	4.5	115
	Y52	0.9	436	32.2	6	3.3	4.3	4.0	147	4.2	117
	Mean	**1.0**	**203.5**	**28.8**	**5.9**	**3.3**	**4.3**	**6.4**	**147.9**	**3.9**	**116.1**
	SD	**0.2**	**95.5**	**5.4**	**1.9**	**0.2**	**0.4**	**1.7**	**1.9**	**0.8**	**1.6**
	P1	1.2	290	27.0	8	3.4	4.6	9.2	149	2.5	117
20 mg/kg	P9	0.6	321	26.6	6	3.9	4.5	5.8	150	4.6	120
	P10	0.8	144	21.1	1	2.8	3.6	6.2	145	3.2	112
	P16	0.5	140	9.8	2	2.4	2.8	11.1	149	3.2	117
	P17	1.0	90	34.7	8	3.7	4.7	5.2	144	2.9	114
	P22	1.3	152	31.4	10	3.3	4.6	11.9	150	3.0	117
	P24	1.1	175	35.7	9	4.1	5.3	7.3	148	3.8	116
	P31	1.1	104	31.2	8	3.2	4.3	5.7	147	2.6	112
	P39	0.9	366	22.9	6	2.9	3.8	4.1	146	2.6	118
	P42	1.0	147	26.2	11	3.4	4.5	7.3	148	3.7	117
	Mean	**1.0**	**192.9**	**26.7**	**6.9**	**3.3**	**4.3**	**7.4**	**147.6**	**3.2**	**116.0**
	SD	**0.3**	**96.4**	**7.6**	**3.2**	**0.5**	**0.7**	**2.6**	**2.1**	**0.7**	**2.6**

	G7	1.0	294	30.1	11	3.3	4.3	5.5	151	3.0	116
	G11	1.0	214	30.1	7	3.3	4.4	3.4	149	4.4	117
100 mg/kg	G14	1.3	141	33.9	3	3.6	4.9	6.9	148	3.1	115
	G15	1.0	121	31.9	16	3.4	4.4	7.3	153	2.8	118
	G18	1.1	314	36.7	6	3.6	4.8	5.0	148	3.4	116
	G26	1.1	136	26.7	6	3.3	4.3	4.6	149	4.3	117
	G30	0.9	195	28.5	9	3.2	4.1	7.1	148	4.3	116
	G31	1.0	126	42.0	9	4.1	5.1	8.7	149	4.3	119
	G35	0.8	471	27.6	3	2.9	3.7	2.6	149	3.7	119
	G38	1.0	117	29.0	6	3.1	4.1	7.8	146	3.7	113
	G40	1.0	172	28.9	5	3.3	4.4	9.5	149	3.5	116
	G42	1.4	167	30.4	8	3.5	4.8	5.8	149	3.8	119
	Mean	**1.1**	**205.7**	**31.3**	**7.4**	**3.4**	**4.4**	**6.2**	**149.0**	**3.7**	**116.8**
	SD	**0.2**	**105.8**	**4.4**	**3.6**	**0.3**	**0.4**	**2.1**	**1.7**	**0.6**	**1.8**

Table P-2
12 Week Individual Clinical Chemistry Analyses
F0 Generation Male Quail

Dose Group	Animal ID	ALB (g/dL)	AST (U/L)	Ca (mg/dL)	GGT (U/L)	GLOB (mg/dL)	TP (mg/dL)	URIC (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
	Y2	0.6	169	9.7	3	2.2	2.8	8.7	152	3.1	118
	Y7	0.7	266	27.4	16	3.0	3.7	8.7	150	3.2	118
Control	Y8	0.6	236	10.0	1	2.5	3.0	7.1	149	4.0	118
	Y10	0.7	214	10.3	2	2.7	3.4	5.7	153	3.2	118
	Y19	0.6	232	9.1	2	2.4	3.0	9.1	145	3.5	116
	Y26	0.6	291	9.4	2	2.5	3.1	2.2	153	3.5	116
	Y35	0.7	302	9.3	7	2.5	3.2	13.4	149	3.1	118
	Y38	1.0	268	10.0	3	2.8	3.8	8.0	147	3.1	117
	Y39	0.7	224	10.2	5	2.5	3.2	10.5	149	3.1	116
	Y42	0.6	222	9.3	3	2.4	3.0	11.1	148	4.0	117
	Y51	0.8	272	10.2	8	2.6	3.4	9.3	153	2.9	120
	Mean	**0.7**	**245.1**	**11.4**	**4.7**	**2.6**	**3.2**	**8.5**	**149.8**	**3.3**	**117.5**
	SD	**0.1**	**38.8**	**5.3**	**4.3**	**0.2**	**0.3**	**2.9**	**2.7**	**0.4**	**1.2**
	P3	0.5	168	9.3	3	2.4	2.9	8.2	149	4.3	117
20 mg/kg	P5	0.7	196	10.3	5	2.8	3.5	7.5	152	3.1	120
	P12	0.6	257	9.5	5	2.6	3.1	13.0	148	3.4	118
	P14	0.7	213	10.3	6	2.6	3.3	10.4	153	3.9	118
	P18	0.9	289	10.0	3	2.6	3.6	6.5	146	3.5	115
	P19	0.6	234	9.8	8	2.6	3.2	5.9	149	3.3	116
	P20	0.4	156	9.9	3	2.3	2.7	14.3	152	3.7	119
	P21	0.7	342	9.8	9	2.4	3.1	7.5	147	2.8	115
	P26	0.8	200	10.0	7	2.7	3.5	10.8	150	3.6	117
	P34	0.5	214	9.9	5	2.6	3.1	8.5	148	4.1	118
	P38	0.8	557	10.5	7	2.9	3.7	10.2	150	4.2	117
	P40	0.6	305	10.5	2	2.6	3.2	7.8	151	4.2	118

	Mean	**0.7**	**260.9**	**10.0**	**5.3**	**2.6**	**3.2**	**9.2**	**149.6**	**3.7**	**117.3**
	SD	**0.1**	**108.6**	**0.4**	**2.2**	**0.2**	**0.3**	**2.6**	**2.2**	**0.5**	**1.5**
	G2	0.6	315	9.6	4	2.4	3.0	4.4	145	3.9	118
	G8	0.8	281	9.3	5	2.6	3.4	9.9	143	2.8	115
100 mg/kg	G9	0.8	379	9.7	3	2.6	3.4	9.1	147	4.7	115
	G17	0.9	129	9.9	9	2.7	3.6	10.7	149	2.8	114
	G19	0.4	161	9.5	2	2.2	2.7	3.3	146	3.6	114
	G20	0.9	298	9.8	9	2.6	3.5	5.6	150	3.1	118
	G24	0.7	252	9.3	3	2.6	3.3	8.8	146	4.9	115
	G27	0.8	183	9.7	8	2.6	3.4	8.7	149	3.6	117
	G28	0.8	257	10.0	4	2.7	3.4	19.2	147	3.5	116
	G32	0.5	178	10.0	5	2.4	2.9	6.8	150	3.7	119
	G39	0.5	248	9.6	6	2.6	3.0	10.1	149	3.5	118
	Mean	**0.7**	**243.7**	**9.7**	**5.3**	**2.5**	**3.2**	**8.8**	**147.4**	**3.6**	**116.3**
	SD	**0.2**	**74.8**	**0.2**	**2.5**	**0.2**	**0.3**	**4.2**	**2.2**	**0.7**	**1.8**

Table P-3
10 Week Individual Clinical Chemistry Analyses
F1 Generation Female Quail

Dose Group	Animal ID	ALB (g/dL)	ALT (U/L)	AST (U/L)	Ca (mg/dL)	GGT (U/L)	GLOB (mg/dL)	TP (mg/dL)	URIC (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
	Y52	1.4	10	267	30.2	3	3.0	4.4	5.0	151	2.9	115
	Y57	1.1	10	762	27.7	2	3.0	4.1	3.7	151	3.7	114
Control	Y64	1.5	10	218	21.8	7	3.2	4.7	10.1	155	3.6	116
	Y65	1.4	10	141	28.6	5	3.0	4.4	6.5	149	4.1	113
	Y67	0.9	10	179	20.3	2	3.3	4.2	5.6	153	3.8	116
	Y70	1.5	10	181	34.9	6	3.6	5.1	7.7	151	4.7	114
	Y73	1.5	12	694	34.2	10	3.5	5.0	5.0	151	4.3	113
	Y78	0.8	10	310	30.4	11	3.6	4.3	1.1	152	3.3	114
	Y80	1.1	10	590	27.5	3	3.2	4.3	2.8	146	3.8	111
	Y82	1.3	10	131	22.9	9	2.6	3.9	2.0	152	3.2	115
	Y83	1.3	10	1139	45.6	3	7.3	8.6	2.8	153	4.7	116
	Y85	1.4	10	93	32.2	8	3.1	4.4	13.0	150	4.0	144
	Y86	0.9	10	170	32.2	2	3.9	4.8	2.7	148	3.6	113
	Mean	**1.2**	**10.2**	**375.0**	**29.9**	**5.5**	**3.6**	**4.8**	**5.2**	**150.9**	**3.8**	**116.5**
	SD	**0.3**	**0.6**	**320.7**	**6.6**	**3.3**	**1.2**	**1.2**	**3.4**	**2.3**	**0.5**	**8.4**
	P52	0.7	10	112	26.9	4	3.0	3.7	7.0	152	5.1	115
20 mg/kg	P54	1.5	10	803	29.1	7	3.3	4.8	7.1	153	3.8	115
	P57	1.2	10	375	31.2	3	2.8	4.0	4.1	152	4.3	115
	P58	0.8	10	414	27.9	0	3.2	4.0	6.0	153	4.9	117
	P60	1.2	10	427	34.2	6	2.8	4.0	4.3	148	4.7	112
	P61	1.3	10	175	31.5	4	3.0	4.3	5.5	153	3.3	114
	P62	1.4	10	252	31.6	11	2.9	4.3	4.0	151	3.6	116
	P65	1.3	10	421	31.9	4	3.3	4.6	4.3	151	3.2	114
	P70	1.4	10	241	34.1	9	3.2	4.6	7.3	148	4.6	112
	P73	1.1	10	325	33.7	6	3.6	4.7	7.2	151	3.8	114

	P77	1.0	21	590	25.3	9	2.6	3.7	2.4	149	4.6	117
	P78	0.7	10	157	28.4	8	3.4	4.1	6.3	151	3.1	114
	P86	1.4	10	381	31.6	3	3.3	4.7	4.9	149	4.2	113
	P89	1.3	10	170	26.8	8	3.0	4.4	5.4	152	2.8	115
	P92	1.4	10	530	38.3	3	3.7	5.0	3.6	153	3.4	118
	P94	1.2	13	114	22.2	7	2.8	4.0	5.4	153	2.7	115
	P95	1.3	10	177	24.7	12	2.8	4.1	7.5	151	3.6	112
	Mean	**1.2**	**10.8**	**333.2**	**30.0**	**6.1**	**3.1**	**4.3**	**5.4**	**151.2**	**3.9**	**114.6**
	SD	**0.2**	**2.7**	**189.0**	**4.1**	**3.2**	**0.3**	**0.4**	**1.5**	**1.7**	**0.7**	**1.8**
	G53	0.4	10	296	22.8	1	2.7	3.1	5.7	147	4.3	114
	G54	1.1	10	334	36.3	0	3.6	4.6	5.5	151	5.7	115
100 mg/kg	G59	1.5	10	806	39.4	7	3.8	5.3	5.7	150	3.6	114
	G63	1.0	10	229	30.6	4	3.7	4.7	2.8	149	2.9	113
	G69	1.5	10	170	35.7	5	3.5	4.9	5.4	151	4.3	116
	G71	1.0	10	270	20.6	7	2.4	3.5	8.5	151	3.2	113
	G72	1.1	10	186	22.7	1	2.3	3.5	3.6	153	3.7	118
	G75	1.2	10	232	33.0	3	2.8	3.9	5.6	152	4.0	114
	G77	1.2	10	239	26.0	4	3.1	4.3	4.2	153	3.1	116
	G78	1.3	10	163	25.7	4	2.7	3.9	5.5	150	5.2	115
	G79	1.5	10	204	25.4	0	3.1	4.6	5.8	154	3.7	115
	G80	1.3	10	148	23.7	2	2.8	4.1	5.3	149	4.3	113
	G82	1.1	15	290	25.3	9	2.7	3.8	3.4	151	3.7	115
	G84	1.3	10	454	28.7	3	2.8	4.1	4.1	151	3.3	115
	G88	1.3	10	671	30.0	8	3.2	4.5	4.8	154	3.4	118
	Mean	**1.2**	**10.3**	**312.8**	**28.4**	**3.9**	**3.0**	**4.2**	**5.1**	**151.1**	**3.9**	**114.9**
	SD	**0.3**	**1.3**	**191.1**	**5.6**	**2.9**	**0.5**	**0.6**	**1.4**	**1.9**	**0.8**	**1.6**

Table P-4
10 Week Individual Clinical Chemistry Analyses
F1 Generation Male Quail

Dose Group	Animal ID	ALB (g/dL)	ALT (U/L)	AST (U/L)	Ca (mg/dL)	GGT (U/L)	GLOB (mg/dL)	TP (mg/dL)	URIC (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
	Y51	1.0	10	154	10.3	7	2.4	3.4	5.8	151	4.1	115
	Y53	0.9	10	162	10.1	3	2.1	3.1	14.0	153	4.2	116
Control	Y54	1.0	10	236	10.3	5	2.3	3.2	13.4	151	3.6	115
	Y58	0.6	10	194	10.1	3	2.7	3.3	10.0	152	3.1	115
	Y60	0.6	10	166	9.7	7	2.7	3.3	8.9	152	3.6	115
	Y63	1.0	10	210	10.0	4	2.1	3.1	9.6	153	3.4	116
	Y66	1.1	10	210	9.9	5	2.2	3.3	4.8	151	4.1	115
	Y69	1.2	10	244	10.2	5	2.5	3.6	7.9	153	4.4	117
	Y71	0.9	10	141	9.7	2	1.9	2.8	11.0	150	4.1	114
	Y72	1.0	10	204	10.0	7	2.2	3.3	10.3	151	4.6	115
	Y74	0.6	10	213	9.5	6	2.7	3.2	8.7	152	3.7	117
	Y81	1.2	10	203	10.3	5	2.4	3.6	4.9	155	3.1	117
	Y84	1.0	10	178	9.8	6	2.2	3.2	7.9	149	3.7	114
	Y87	1.0	10	219	10.5	3	2.2	3.2	5.6	156	3.7	120
	Y88	1.2	10	217	10.2	7	2.5	3.7	12.2	154	3.6	117
	Mean	**1.0**	**10.0**	**196.7**	**10.0**	**5.0**	**2.3**	**3.3**	**9.0**	**152.2**	**3.8**	**115.9**
	SD	**0.2**	**0.0**	**30.3**	**0.3**	**1.7**	**0.2**	**0.2**	**2.9**	**1.9**	**0.4**	**1.6**
	P51	1.0	10	169	9.9	6	2.2	3.3	9.1	155	2.5	114
20 mg/kg	P59	1.1	10	161	10.3	3	2.2	3.2	4.0	152	4.7	116
	P63	1.0	10	214	10.1	4	2.1	3.1	5.3	153	2.3	117
	P67	1.1	10	202	9.7	3	2.2	3.3	2.7	151	4.3	116
	P68	0.4	10	177	9.6	6	2.5	2.9	11.9	152	4.0	115
	P69	0.5	10	187	9.6	2	2.6	3.1	11.5	151	4.5	115
	P72	1.2	10	169	10.0	3	2.5	3.8	9.9	153	3.6	113
	P74	1.1	10	194	9.8	5	2.2	3.3	17.7	154	4.3	115

	P75	0.9	10	223	9.7	2	2.0	2.9	5.4	153	3.8	118
	P79	1.2	10	163	10.3	0	2.3	3.4	9.2	156	2.6	116
	P80	1.0	10	192	9.9	5	2.1	3.1	7.8	152	4.2	115
	P81	1.0	10	176	9.7	6	2.2	3.3	7.8	150	3.9	115
	P84	1.2	10	212	10.3	4	2.5	3.7	8.0	151	4.4	115
	P90	0.9	10	213	9.4	9	2.0	2.9	5.5	153	4.0	117
	P91	1.1	10	307	9.6	2	2.1	3.2	10.2	151	3.1	111
	P96	1.1	10	201	9.8	6	2.3	3.3	5.9	153	3.7	117
	P98	1.2	10	198	10.1	5	2.5	3.7	14.9	155	3.8	115
	P99	0.5	10	234	9.9	6	2.5	3.0	8.2	153	3.6	116
	Mean	**1.0**	**10.0**	**199.6**	**9.9**	**4.3**	**2.3**	**3.3**	**8.6**	**152.7**	**3.7**	**115.3**
	SD	**0.3**	**0.0**	**34.2**	**0.3**	**2.1**	**0.2**	**0.3**	**3.8**	**1.6**	**0.7**	**1.6**
	G52	1.1	10	175	10.0	5	2.3	3.3	13.1	156	3.3	118
	G55	1.2	10	163	9.8	4	2.3	3.5	10.8	149	4.5	113
100 mg/kg	G57	0.6	10	240	9.9	4	2.6	3.2	11.7	152	3.5	115
	G58	0.9	10	194	9.8	3	2.1	3.0	10.7	153	2.8	116
	G62	1.0	10	180	9.8	9	2.3	3.3	4.9	153	3.1	116
	G64	1.0	10	172	9.6	4	2.2	3.3	15.3	151	4.1	114
	G65	1.0	10	185	9.5	7	2.0	3.0	5.3	153	3.4	117
	G66	0.7	10	179	9.8	2	2.6	3.3	10.8	154	4.3	117
	G68	0.5	10	247	9.6	1	2.6	3.1	8.8	150	4.9	115
	G74	0.9	10	184	10.2	2	2.0	2.9	11.0	154	4.4	118
	G76	1.0	10	161	9.8	3	2.3	3.3	9.9	154	3.4	115
	G81	1.2	10	243	9.7	6	2.3	3.5	8.2	153	3.7	115
	G85	1.1	10	179	9.9	3	2.4	3.5	10.5	152	3.9	116
	G86	1.2	10	207	10.2	6	2.3	3.5	7.0	156	3.0	118
	G87	0.5	10	160	9.4	3	2.6	3.1	8.0	155	3.1	117
	G90	1.0	10	188	9.4	4	2.1	3.1	11.8	151	3.2	116
	Mean	**0.9**	**10.0**	**191.1**	**9.8**	**4.1**	**2.3**	**3.2**	**9.9**	**152.9**	**3.7**	**116.0**
	SD	**0.2**	**0.0**	**28.6**	**0.2**	**2.1**	**0.2**	**0.2**	**2.7**	**2.0**	**0.6**	**1.5**

Appendix Q

Individual and Summary Hematology Data

Table Q-1
12 Week Individual Hematology Analyses
F0 Generation Female Quail

Dose Group	Animal ID	Hgb (g/dL)	Hematocrit (%)	Total Solids (g/dL)
	Y1	13.7	39	5.7
	Y4	12.7	37	5.0
Control	Y11	14.5	41	5.7
	Y22	13.1	38	6.0
	Y23	14.5	42	6.7
	Y24	13.7	41	6.1
	Y29	14.7	41	7.7
	Y30	16.1	45	4.8
	Y34	18.2	51	5.0
	Y46	13.8	38	6.3
	Y52	13.5	38	6.2
	Mean	**14.4**	**41.0**	**5.9**
	SD	**1.6**	**4.0**	**0.8**
	P1	14.3	42	5.5
	P9	13.0	37	10.0
20 mg/kg	P10	15.0	43	4.7
	P16	18.8	50	3.4
	P17	15.5	44	6.7
	P22	15.1	43	5.8
	P24	15.7	45	8.1
	P31	15.4	44	5.5
	P39	13.8	38	5.3
	P42	15.2	43	6.5
	Mean	**15.2**	**42.9**	**6.2**
	SD	**1.5**	**3.6**	**1.8**
	G7	14.4	41	6.0
	G11	15.2	43	6.0
100 mg/kg	G14	14.5	42	6.4
	G15	15.2	43	6.1
	G18	12.9	37	6.9
	G26	16.1	46	5.8
	G30	16.5	45	5.4
	G31	13.6	40	7.5
	G35	14.0	40	5.2
	G38	13.3	39	5.8
	G40	15.3	44	5.6
	G42	15.4	43	5.9
	Mean	**14.7**	**41.9**	**6.1**
	SD	**1.1**	**2.6**	**0.6**

Table Q-2
12 Week Individual Hematology Analyses
F0 Generation Male Quail

Dose Group	Animal ID	Hgb (g/dL)	Hematocrit (%)	Total Solids (g/dL)
	Y2	17.5	47	3.6
	Y7	14.6	40	4.7
Control	Y8	18.4	50	4.0
	Y10	19.1	53	4.1
	Y19	17.9	50	3.7
	Y26	17.0	46	3.9
	Y35	17.4	48	3.8
	Y38	18.5	52	4.4
	Y39	17.9	48	3.8
	Y42	19.0	53	4.0
	Y51	19.8	53	4.0
	Mean	**17.9**	**49.1**	**4.0**
	SD	**1.4**	**3.9**	**0.3**
	P3	18.7	50	3.6
	P5	17.2	48	4.3
20 mg/kg	P12	17.8	49	4.0
	P14	18.6	49	4.0
	P18	18.8	51	4.2
	P19	17.9	48	3.8
	P20	18.4	48	3.4
	P21	17.6	48	3.8
	P26	17.6	48	4.2
	P34	18.9	50	3.5
	P38	20.4	54	4.5
	P40	19.3	ND	3.9
	Mean	**18.4**	**49.4**	**3.9**
	SD	**0.9**	**1.9**	**0.3**
	G2	18.8	51	3.7
	G8	19.3	52	4.0
100 mg/kg	G9	18.3	50	4.3
	G17	18.7	51	4.3
	G19	17.5	48	3.5
	G20	18.3	50	4.0
	G24	18.8	50	3.9
	G27	18.7	51	4.2
	G28	19.1	51	4.5
	G32	18.7	50.0	3.8
	G39	19.7	53	3.8

	Mean	**18.7**	**50.6**	**4.0**
	SD	**0.6**	**1.3**	**0.3**
500 mg/kg	B18	16.1	46	4.3

Table Q-3
10 Week Individual Hematology Analyses
F1 Generation Female Quail

Dose Group	Animal ID	Hgb (g/dL)	Hematocrit (%)	Total Solids (g/dL)
	Y52	15.2	46	6.0
	Y57	14.3	41	7.2
Control	Y64	19.1	53	5.9
	Y65	15.3	45	5.8
	Y67	14.1	41	5.5
	Y70	14.8	44	7.4
	Y73	13.9	43	6.9
	Y78	14.3	41	7.5
	Y80	11.8	35	8.1
	Y82	16.2	49	4.8
	Y83	12.5	38	13.3
	Y85	15.4	46	6.0
	Y86	14.2	ND	7.5
	Mean	**14.7**	**43.5**	**7.1**
	SD	**1.8**	**4.8**	**2.1**
	P52	14.5	40	5.3
	P54	13.6	42	6.2
20 mg/kg	P57	14.1	42	5.4
	P58	13.1	37	6.0
	P60	13.1	40	5.9
	P61	16.9	41	6.0
	P62	13.9	42	5.9
	P65	13.3	40	7.7
	P70	14.1	41	5.6
	P73	16.3	46	6.2
	P77	12.4	38	5.8
	P78	15.4	44	6.1
	P86	15.0	45	6.7
	P89	16.4	48	6.0
	P92	12.9	38	7.4
	P94	14.7	43	4.8
	P95	14.0	42	5.0
	Mean	**14.3**	**41.7**	**6.0**
	SD	**1.3**	**2.9**	**0.7**
	G53	14.3	40	4.2
	G54	15.6	43	6.4
100 mg/kg	G59	16.2	48	7.5
	G63	15.6	45	6.4
	G69	14.0	40	6.7

G71	15.3	46	4.4
G72	13.3	40	4.3
G75	13.1	40	5.6
G77	14.9	44	6.0
G78	13.6	40	4.9
G79	13.3	38	5.5
G80	13.7	42	5.2
G82	14.3	42	5.3
G84	13.4	40	5.4
G88	12.5	39	6.5
Mean	**14.2**	**41.8**	**5.6**
SD	**1.1**	**2.9**	**1.0**

Table Q-4
10 Week Individual Hematology Analyses
F1 Generation Male Quail

Dose Group	Animal ID	Hgb (g/dL)	Hematocrit (%)	Total Solids (g/dL)
	Y51	17.9	50	4.2
	Y53	18.0	52	3.7
Control	Y54	18.8	53	3.8
	Y58	19.5	52	3.9
	Y60	18.4	50	4.1
	Y63	17.0	40	3.9
	Y66	16.0	47	4.1
	Y69	17.7	51	4.4
	Y71	18.7	49	3.4
	Y72	19.3	56	3.8
	Y74	18.3	50	4.0
	Y81	18.4	52	4.6
	Y84	19.5	55	3.8
	Y87	18.4	53	4.0
	Y88	19.6	56	4.4
	Mean	**18.4**	**51.1**	**4.0**
	SD	**1.0**	**4.0**	**0.3**
	P51	18.2	51	4.3
	P59	17.7	50	3.8
20 mg/kg	P63	17.2	49	3.7
	P67	17.8	52	4.0
	P68	16.5	44	3.6
	P69	19.0	50	3.8
	P72	16.8	49	4.3
	P74	18.1	51	4.0
	P75	18.0	50	3.6
	P79	18.6	53	4.3
	P80	13.7	48	3.8
	P81	18.6	53	3.9
	P84	18.1	52	4.3
	P90	17.2	50	3.7
	P91	18.6	52	3.9
	P96	17.7	50	3.8
	P98	17.1	49	4.5
	Mean	**17.7**	**50.4**	**3.9**
	SD	**1.4**	**2.3**	**0.3**
	G52	18.0	51	4.0
	G55	16.6	48	4.2
100 mg/kg	G57	18.6	50	4.0

G58	16.7	48	3.7
G62	19.1	55	3.7
G64	17.8	50	4.0
G65	18.8	53	3.8
G66	19.2	52	4.0
G68	16.1	45	3.8
G74	16.4	47	3.6
G76	17.6	51	4.2
G81	19.0	53	4.4
G85	17.7	51	4.4
G86	18.5	53	4.2
G87	17.4	48	3.8
Mean	**17.8**	**50.2**	**4.0**
SD	**1.0**	**2.7**	**0.2**

Appendix R

Study Protocol with Modifications

ANIMAL USE PROTOCOL
U.S. ARMY PUBLIC HEALTH COMMAND
ABERDEEN PROVING GROUND. MD 21010-5403

PROTOCOL TITLE: One-generation reproductive toxicity test in Japanese quail *(Coturnix japonica)* using 3-nitro-1,2,4-triazol-5-one (NTO)

PROTOCOL NUMBER: 80-14-07-02

DATE OF APPROVAL: 03 JULY 2014

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ACRONYMS:

AAALAC: Association for Assessment and Accreditation of Laboratory Animal Care International
ANCOVA: Analysis of Covariance
ANOVA: Analysis of Variance
AV: Attending Veterinarian
BRD: Biomedical Research Database
CO_2*:* carbon dioxide
DOD: Department of Defense
DTIC: Defense Technical Information Center
ED: embryonic day
EDC: endocrine disrupting chemical
ESTCP: Environmental Security Technology Certification Program
F0: parental generation
F1: first generation
FEDRIP: Federal Research in Progress Database
IACUC: Institutional Animal Care and Use Committee
IAW: in accordance with
IM: insensitive munitions
IP: intraperitoneal
IV: intravenous
LD_{50}: median lethal dose
LS: Laboratory Sciences Portfolio
ml: milliliter
mg/kg: milligram per kilogram
mg/kg-day: milligram per kilogram per day
NTIS: National Technical Information Service
NTO: 3-nitro-1,2,4-triazol-5-one
OECD: Organisation for Economic Co-Operation and Development
OHS: Occupational Health and Safety
PBS: phosphate buffered saline
PPE: personal protective equipment
QC: quality control
RBC: red blood cell
RDX: Research Development Explosive or Royal Demolition Explosive
SERDP: Strategic Environmental Research and Development Program
SOP: Standing Operating Procedure
SD/PI: Study Director/Principal Investigator
TOX: Portfolio of Toxicology
TNT: trinitrotoluene
TRV: toxicity reference value
US EPA: United States Environmental Protection Agency
USACHPPM: United States Army Center for Health Promotion and Preventive Medicine
USAPHC: United States Army Public Health Command
VMD: Veterinary Medical Division

I. NON-TECHNICAL SYNOPSIS: NTO is being investigated as a less sensitive replacement for traditional explosives such as TNT and RDX. NTO must not only meet certain performance criteria, but must also be acceptable from the perspective of human health and the environment. Prior data suggests that NTO may cause problems in the endocrine system, therefore; this study will assess the reproductive and developmental toxicity of NTO in Japanese quail. First, a group of birds will be used to determine the doses for a one-generation study. Then, the one-generation study will be conducted: eggs will be set and incubated, and birds that hatch will be grown and given various doses of NTO orally. The effects on their body weight and behavior, as well as effects on reproductive and immunological parameters, will be evaluated.

II. BACKGROUND

II.1. Background:

Acute toxicity testing of NTO demonstrated that NTO has low toxicity (LD_{50} > 5g/kg) in rats and mice (London and Smith 1985). Subacute and subchronic oral studies in rats demonstrated limited hematological effects (slight anemia) and liver hyperplasia/hypertrophy only in doses at or greater than 1000 mg/kg-day NTO. The most pronounced effects of NTO exposure were testicular and epididymal toxicity and hypospermia (Crouse et al. 2010).

To determine whether the testicular toxicity of NTO is indicative of endocrine disrupting effects or direct testicular toxicity, both a reproductive/developmental screening test and a battery of *in vivo* endocrine disruptor screening tests were conducted by USAPHC. Results from these screening studies suggest that at doses between 31.25 and 500 mg/kg-day administered for two weeks pre-mating, NTO did not affect mating or pregnancy rate in rats (Crouse et al. 2010).

The Hershberger and uterotrophic assays did not demonstrate anti-androgenic or estrogenic activity, respectively, for NTO at doses up to 1000 mg/kg-day. NTO had no effect on timing of pubertal development in the male and female pubertal developmental and thyroid function assays. In females, there was no effect on tissue mass; however, in males, significant reductions in the mass of the testes and epididymides were observed. Testis mass was reduced to 70% and 35% of control in the 250 and 500 mg/kg-day groups ($p<0.001$), while epididymides were reduced to 76% of control in the 500 mg/kg-day group ($p \leq 0.001$) (Quinn et al. 2012). These preliminary results may indicate antiandrogenic activity or effects on steroidogenesis; however, direct testicular toxicity is possible given the lack of effects on pubertal timing. The limited effects on accessory tissues may be secondary to testicular toxicity and impaired testicular endocrine function (Lent et al. in prep.).

An extended one-generation study in rats was recently completed to bridge the gaps between previously conducted studies by evaluating specific life states not covered by other types of studies and testing for effects that may occur as a result of combined pre- and postnatal exposure (Lent et al, in prep). Although analysis is incomplete, preliminary results suggest an absence of testes and sperm effects, as have been

observed in the previously discussed studies. The main difference in study methods between the Crouse and Lent studies is in oral exposure methods and the doses received as a result of the exposure method; Crouse et al. (2010) exposed rats to NTO via daily gavages while Lent et al. (in prep.) dosed rats with NTO in drinking water. Based on water consumption calculations from the drinking water study, it was realized that some rats in the Lent et al. study received lower doses than intended. It is speculated that this occurred because of an aversion to drinking NTO, further supporting the need for an avian gavage study with NTO.

The current study will expose hatched quail to NTO in daily gavages. Birds will be tested for reproductive effects by examining body weight, behavior and mating, sexual development and fertility, etc. Particular attention will be paid to determine if male reproductive effects are conserved among species. The study will include a high concentration recovery group to see whether partial or full recovery of testicular mass occurs. Additionally, NTO will be investigated for immunological effects via a foreign RBC challenge. The addition of avian data will help to reduce the uncertainty factors used in TRVs developed for human health assessments; if NTO does not appear to act as an EDC in rats or birds, there is a greater chance of the compound not acting as one in humans. Avian specific data has other merits as past regulatory concerns for threatened and endangered bird species, bald eagles, and other protected migratory bird species at installations suggest the need for collection of class specific (Aves) toxicity data.

II.2. Literature Search for Duplication

II.2.1. Literature Source(s) Searched: BRD, DTIC, NTIS, PubMed, Web of Science

II.2.2. Date of Search: 26 March 2014

II.2.3. Period of Search: All years covered by databases

II.2.4. Key Words of Search:
#1: (3-nitro-1,2,4-triazol-5-one or 3 nitro 1,2,4 triazol 5 one or triazoles or nitro compounds) and ("endocrine disruption" or "endocrine disrupting chemical") and (japanese quail or *Coturnix japonica*)

#2: and (japanese quail or *Coturnix japonica*) and ("endocrine disruption" or "endocrine disrupting chemical")

#3: (3-nitro-1,2,4-triazol-5-one or 3 nitro 1,2,4 triazol 5 one or triazoles or nitro compounds) and ("endocrine disruption" or "endocrine disrupting chemical")

II.2.5. Results of Search: A total of 110 references resulted from the literature search that was performed using the search strategies and key words listed above in all the listed databases. However, no studies were found that would suggest that this study would be a duplicate effort. As such, the present study is not a duplication of the information available in the literature.

III. OBJECTIVE/HYPOTHESIS: The main objective of the one-generation reproductive toxicity test is to evaluate whether 3-nitro-1,2,4-triazol-5-one (NTO) is an EDC in Japanese quail (*Coturnix japonica*). Additionally, NTO will be investigated for immunological effects.

IV. MILITARY RELEVANCE: NTO is being investigated as a less sensitive (i.e. more resistant to accidental explosion), direct replacement for traditional explosives such as TNT and RDX. As a potential component of new munitions formulations, NTO must not only meet certain performance criteria, but must also be acceptable from the perspective of human health and the environment. Prior data suggests that NTO may be an EDC; this study will assess NTO's potential as an EDC to protect Soldiers and public health. The EPA will likely regulate NTO at some point and the Army is proactively assembling the supporting studies that will ensure realistic environmental regulation. The results from this bird study will support wildlife toxicity assessment as well as human risk assessment.

V. MATERIALS AND METHODS

Test Article: This study will be conducted with NTO. NTO is obtained from BAE Systems, Ordnance Systems Kingsport, TN; Batch 10NTO-3, Lot BAE07B301-001. NTO will be mixed with corn oil. Samples of each batch of the resulting dosing solutions will be submitted to LS for concentration verification. Neat test material will be stored at room temperature (20±5ºC). Neat test material may be stored in anti-static bags or sample jars and may be stored in a desiccator to reduce contamination with moisture. Sample analysis will be done IAW SOP DLS 801.2 (USAPHC 2014a).

Table 1. Test Substance Chemical/Physical Properties

Name	3-nitro-1,2,4-triazol-5-one
Synonym	NTO
CAS#	932-64-9
Physical State	White to pale yellow crystalline powder
Molecular Formula	$C_2H_2N_4O_3$
Molecular Weight	130
Density	1.93 g/cm^3
Solubility	Soluble in water (16 g/L)

V.1. Experimental Design and General Procedures: The reproductive and developmental toxicity of NTO, will be assessed using a modified Avian Reproductive Test (OECD 1984). This study will evaluate whether or not NTO is an EDC. Effects of embryonic and post-hatch exposure to NTO on development and systemic toxicity in adults and offspring will be evaluated, as well as whether NTO has similar effects to those seen in more traditional laboratory species (i.e. rats and mice).

An Avian Acute Oral Toxicity Test will be conducted prior to starting the one-generation study. In the one-generation study, quail will be given NTO in corn oil at four

concentrations and a vehicle control. There will also be a group of birds exposed at the highest concentration and then taken off treatment to see if partial or full recovery of testicular mass occurs. All birds will be monitored throughout the study for body weight changes and clinical signs of toxicity. The experiment is estimated to begin in September 2014 and end in February 2015.

Table 2. Number of eggs to be set and quail to be used in study

Group	No. Quail		Pain Category
Avian Acute Oral Toxicity Test			
Limit dose test	*20 max*		10 C / 10 E
Stage 1. Sequential design (if necessary)	*14*		10 C / 4 E
Stage 2. Sequential design (if necessary)	*20*		10 C / 10 E
	TOTAL = *54*		**30 C / 24 E**
One-Generation			
Estimated F0 eggs set			
5 Groups; 60 eggs each	5 x 60 = **300 Eggs**		
Estimated F0 chicks produced	**Males**	**Females**	**Pain Category**
5 Groups; ~70% hatch rate w/ 1:1 sex ratio; (300 eggs x 0.7) / 5 groups x (0.5 sex) = 21 each sex per group	5 x 21 = **105**	5 x 21 = **105**	
F0 generation *12 of 21 assigned to Dose Group*	*5 x 12 = 60*	*5 x 12 = 60*	120 C
4 of 21 reserved as Replacements	*5 x 4 = 20*	*5 x 4 = 20*	40 C
0-1 of 21 used as Sentinels	*2*	*2*	4 B
2-3 of 21 assigned to Recovery	*12*	n/a	12 C
Estimated F0 chicks culled 1-3 of 21 males culled 4 of 21 females culled	11	23	34 C
Totals = *Note: Italics indicates birds identified by cage card*	***94* of ~105 on-study**	***82* of ~105 on-study**	**~206 C / 4 B**
Estimated F1 eggs set			
5 Groups; 80 eggs each	5 x 80 = **400 Eggs**		
Estimated F1 chicks produced	**Males**	**Females**	**Pain Category**
5 Groups; ~70% hatch rate w/ 1:1 sex ratio; (400 eggs x 0.7) / 5 groups x (0.5 sex) = 28 each sex per group	5 x 28 = **140**	5 x 28 = **140**	
F1 generation *12 of 28 assigned to Dose Group*	*5 x 12 = 60*	*5 x 12 = 60*	120 C
Replacements; *4 of 28 reserved as Replacements*	*5 x 4 = 20*	*5 x 4 =20*	40 C
Estimated F1 chicks culled 12 of 28 culled	5 x 12 = 60	5 x 12 = 60	120 C
Totals=	***80* of ~140**	***80* of ~140**	**~280 C**

Note: Italics indicates birds identified by cage card	**on-study**	**on-study**	
		Total =	**~516 C, 4 B, 24 E**

V.1.1. Avian Acute Oral Toxicity Test: As acute toxicity testing of NTO demonstrates that NTO has low toxicity (LD_{50} > 5g/kg) in rats and mice, the evaluation will begin with a Limit dose test in quail, and progress to the more extensive, sequential stage testing procedures if necessary (OECD 2009). Birds should be in mature plumage but not in breeding condition, according to OECD guidelines, therefore; birds will most likely be between 4 and 6 weeks of age at the start of the Limit dose test. Five birds will be tested at the limit dose, in addition to a control group consisting of 5 birds. This is the recommended strategy for testing materials that are unlikely to present a significant hazard. After receiving a single dose as described in section V.4.4.8.1, the birds will be observed for a 14-day period in order to measure mortality. Mortality is the primary endpoint in this study and background mortality is presumed to be negligible (OECD 2009). Dosed birds will be euthanized at the conclusion of the Avian Acute Oral Toxicity Test (roughly 6 to 8 weeks of age). Birds that do not receive NTO (those that are held but not used due to the outcome of the acute testing stages) may be transferred to the training protocol (see section V.4.5.).

Table 3: Limit dose test and Sequential design procedures

Limit dose test	
Initially 5 birds at 1 dose and 5 control birds; possibly 5 additional dosed birds and possibly 5 additional controls* (N=20 max).	To determine >limit or estimate initial LD50 for Sequential Design
# Deaths in Dosed Birds	***Response***
5	Proceed to Stage 1. Sequential design.
2-4	Proceed to Stage 2. Sequential design
1	1) If survivors show signs of toxicity, proceed to Stage 2. 2) If survivors show no signs of toxicity *either*: a. Proceed to Stage 2. ***Or*** b. **Treat 5 additional birds** at dose limit. i. If no deaths: **STOP** – LD50 greater than limit determined based on 1/10. ii. If a death occurs proceed to Stage 2.
0	**STOP** – LD50 greater than limit determined.
Stage 1. Sequential design (Up-Down dosing)	
4 birds at 4 doses and 5 control birds; possibly 5 additional controls*. (N=14	Estimate Working LD50 and Proceed to Stage 2.

max).	
Stage 2. Sequential design (Up-Down dosing)	
10 birds at 10 doses and 5 control birds; possibly 5 additional controls* (N=20 max)	If only working LD50 is needed – **STOP**.

* An additional 5 controls will be added to the test in the event of one incidental death in the initial control group.

According to OECD guidelines, animals obviously in pain or showing signs of severe distress will be euthanized. Depending on observed mortalities, further tests comprised of sequential stages could be initiated. In such cases, it is not necessary to wait 14 days before starting the next test, although observation of all birds should continue. Data collected during the first three days is generally sufficient information to determine whether birds are likely to recover from effects encountered, or whether additional mortality will occur.

If there is one incidental death in the initial five-bird control group, then five more control birds are added to the initial test, for a total of 10 control birds, IAW OECD guidelines. The test is invalid if there is one non-incidental death or more than one death from any other cause in the control group, according to OECD guidelines. As many as 54 birds could be used for the Avian Acute Oral Toxicity Test and as many as 30 birds would be control animals. The test ends when birds are euthanized.

V.1.1.1. Dose Selection: According to OECD guidelines, 2000 mg/kg will be used for the Limit dose test of the Avian Acute Oral Toxicity Test. If it is necessary to proceed to a sequential design, doses will be based on results from the Limit dose test and calculations outlined in the OECD guideline for the Avian Acute Oral Toxicity Test.

V.1.1.2. Administration of Test Substance: Birds will receive a single dose of NTO or corn oil vehicle control (as described in section V.4.4.8.1.).

V.1.1.3. Observations: Birds will be observed continuously during the first two hours after dosing for regurgitation and for the onset of clinical signs, on at least three evenly spaced additional occasions during the day for clinical signs, and at least daily thereafter for a total of 14 days. Observations on each individual include regurgitation, signs of intoxication and remission, abnormal behavior, change in body weight, mortality, and time to death. Observations of deaths that are clearly not treatment related should be excluded from calculations (OECD 2009). Animals will be observed for moribundity as described in section V.5.2.1.

V.1.1.4. Body Weight: Animals will be weighed as described in section V.1.2.4.

V.1.2. One-generation study:

To produce the F0 generation, 300 eggs will be set to hatch, as shown in Table 2. Twelve males and 12 females are needed for each treatment group (60 total males and 60 total females), in addition to 8 potential replacements for each treatment group (4 males, 4 females; 40 total). Previous studies have shown that some birds are unable to adapt to the automatic watering system upon their transition from the chick housing to adult caging at approximately 4 weeks of age. Although this inability to learn how to use the automatic watering system is minimized by moving the birds to the new caging as pairs, birds that are unable to make the transition will be replaced with those who can so that an adequate number of birds can achieve reproductive maturity in order to produce the subsequent generation. A group of 12 birds from the F0 generation will be exposed at the highest concentration and then taken off treatment to see if partial or full recovery of testicular mass occurs. An additional 4 birds (2 males and 2 females, if possible) will be kept as sentinels, per VMD SOP 007.000, due to limited vendor history and health status of animals. Sentinels are considered for studies lasting more than 90 days and will be maintained through the conclusion of the study. The remaining hatched chicks will be culled. Termination of the F0 generation adults will occur after behavioral assessment/mating to produce eggs for the F1 generation (at approximately 12 weeks of age).

To generate the F1 generation, 400 eggs produced by the F0 generation will be set to hatch, as shown in Table 2. Twelve males and 12 females are needed for each treatment group (60 total males and 60 total females), in addition to 8 potential replacements for each treatment group (4 males, 4 females; 40 total). The remaining hatched F1 chicks will be culled. Termination of the F1 generation adults will occur after behavioral assessment (at approximately 10 weeks of age). At this time, Recovery males from the F0 generation will also be euthanized (at approximately 23 weeks of age).

Table 4. Experimental design for One-generation study

	Pre-Exposure	Pre-Mating Exposure	Mating Exposure	Post-Mating Exposure
F0 Males	1 week	~7 weeks	~4 weeks	0 weeks
F0 Females	1 week	~7 weeks	~4 weeks	1 week
	Pre-Exposure	Exposure		Recovery
Recovery	1 week	~ 12 weeks		~ 10 weeks
	In Ovo Exposure	Pre-Mating Exposure	Mating Exposure	Post-Mating Exposure
F1 Males	16-18 days	~8 weeks	~2 weeks	0 weeks
F1 Females	16-18 days	~8 weeks	~2 weeks	1 week

V.1.2.1. Dose Selection: Dose selection is based on the ultimate objective of being able to detect reproductive, developmental, and immunotoxic effects, if present, in the one-generation study. To that end, it is recommended that “the highest dose should be chosen with the aim of inducing some systemic toxicity, but not death or severe suffering of the animals” (OECD 2011). In the subacute and subchronic toxicity studies in rats, half the limit dose (1000 mg/kg-day) produced minimal systemic toxicity. Testicular toxicity was the primary effect in rats, occurring at doses as low as 315 mg/kg-day in the 90-day study and 500 mg/kg-day in the 14-day study. The doses for

the one-generation study will be based on the toxicity observed in the Avian Acute Oral Toxicity Test, but are anticipated to be approximately 1000, 500, 100, and 20 mg/kg-day. The low, medium-low, and medium-high doses are set at five fold intervals. The high dose is twice the medium-high dose, as 2000 mg/kg is the limit dose (i.e. we cannot dose at 2500 mg/kg). Doses will be set in consultation with the statistician based on the outcome of the Avian Acute Oral Toxicity Test.

V.1.2.2. Administration of Test Substance: For the F0 generation, we will begin NTO exposure (as described in section V.4.4.8.1.) prior to animals reaching reproductive maturity (at approximately two weeks of age). Exposure will continue until termination when the birds are approximately 12 weeks of age. NTO will be administered 7-days/week. Administration of NTO will cease for recovery group males when F0 males are euthanized, however; this group will be maintained and euthanized with the F1 males in order to observe whether partial or full recovery of testicular mass occurs.

For the F1 generation, NTO exposure will begin *in ovo* via maternal deposition. At day 2 of age, we will begin oral exposure to NTO as described in section V.4.4.8.1. Exposure will continue until termination at approximately 10 weeks of age. NTO will be administered 7-days/week.

V.1.2.3. Observations: A thorough physical examination of each animal will be performed by study personnel at least once per day. The examination process will consist of each animal being removed from its home cage and may be done in conjunction with dosing. Animals will be observed for moribundity as described in section V.5.2.1.

V.1.2.4. Body Weight: F0 animals will be weighed at the start of test compound administration, at least weekly thereafter, and at termination. F1 animals will be weighed on days 1, 3, 7, at least weekly thereafter, and at termination.

V.1.2.5. Assessment of Sexual Development/Fertility: Quail will be examined daily (starting at approximately 4 weeks of age) for presence of foam and date of first egg. Cloacal gland development will be measured as described in section V.4.4.8.2.

V.1.2.6. Behavioral Assessment/Mating: When 90% of control males have reached reproductive maturity (approximately six-eight weeks of age), male copulatory behavior will be assessed (as described in section V.4.4.7.) Male copulatory behavior is a sensitive measure of endocrine disruption. After behavioral assessment, male and female quail will be paired regularly (Monday, Wednesday, and Friday, if possible) to produce the F1 generation. Behavioral assessment and mating will be done in the same manner for the F1 generation; however, a F2 generation will not be produced.

V.1.2.7. Eggs: After behavioral assessment and when at least 90% of control females have reached reproductive maturity, eggs will be collected for multiple evaluation scenarios. Groups of eggs produced by the F0 generation will be processed one of the

following ways: (1) collected, set, artificially incubated, and allowed to hatch to establish the F1 generation; (2) collected, set, and artificially incubated until ED 4 to assess fertility; (3) collected to determine maternal deposition of the chemical; or (4) collected to assess eggshell thickness.

Groups of eggs produced by the F1 generation will be: (1) collected, set, and artificially incubated until ED 4 to assess fertility; (2) collected to determine maternal deposition of the chemical; or (3) collected to assess eggshell thickness.

Ideally, at least one egg will be collected from each female for each evaluation scenario.

V.1.2.8. Immunotoxicity Testing: Immunotoxicity will be evaluated via a foreign RBC challenge (Wright State University, 2001). Injections will be performed as described in section V.4.4.1.1. Blood will be collected (as described in section V.4.4.3.) prior to the injection (as a baseline) and 4-6 days after the injection to measure antibody response. This is an opportunistic study, in which all birds will be evaluated via a foreign RBC challenge rather than using an additional group of birds. Immunotoxicity testing will occur at least two weeks after the start of treatment in both the F0 and F1 generation. Testing will be done with males and females.

V.2. Sample Size Evaluation, Data Analysis Plan, and Archiving of Data:

V.2.1. Sample Size Evaluation: The sample sizes for the study were determined to help ensure that statistical tests will be powerful enough to detect treatment-related differences and to provide rigorous data necessary for TRV derivation (i.e. using benchmark dose algorithm). The number of eggs set to hatch in this study reflects the hatch rate (70%) previously seen at USAPHC from eggs obtained from Lake Cumberland Game Farm, and the number of birds expected to meet the requirement for mating pairs, which should be met based on previously observed sex ratios. Using a hatch rate of 70% is protective; eggs obtained from alternate sources, such as the University of Maryland, have hatched at rates as high as 90%. The number of breeding pairs for the current study is a reduction from the 20 breeding pairs that the US EPA recommends, and is based on the higher breeding rates seen in three similar EDC studies at USAPHC. The number of potential replacements has also been reduced.

V.2.2. Data Analysis Plan: Data will be analyzed using ANOVAs and ANCOVAs for continuous outcome data such as body weight, organ weight, etc. Categorical outcomes (i.e. egg counts, sexual development, etc.) will be analyzed using Chi-square, Fisher's Exact Test, or even Generalized Linear Models when applicable. Time to event outcomes such as days until reproductive maturity, time to mount, etc. will also be investigated using survival analysis techniques. All models will be checked for their appropriate assumptions and accounted for if necessary. Statistical significance will be defined as $p \leq 0.05$ for all tests.

V.2.3. Archiving Data: All observational data will be recorded and kept in standard USAPHC laboratory notebooks and/or three ring binders. Daily records will be kept on survival and clinical signs collected on the animals. Procedures for preparation of any euthanasia solution, drug administration, injections, blood collection, observational logs, morbidity/mortality logs, etc. will be stored. These records will be made available to oversight organizations such as the US EPA, AAALAC, QC and the IACUC. The protocol, protocol amendments, raw data, statistical analysis, tabular calculations, and graphic analysis of the data will be saved for study records. Additionally, memoranda to the study file, study logs, signature logs, final report, and final report amendments will be archived at USAPHC. Some ancillary records such as maintenance and calibration logs, environmental monitoring logs, animal room husbandry and health rounds sheets, all veterinarian staff duties logbooks, training files, etc. may be stored in the archives but not stored with the study files.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered: There are no appropriate animal substitutes (e.g. computer models, tissue/cell cultures) for the data that will be produced in this study. No non-animal alternatives would provide the necessary toxicological information provided by this study. Therefore, it is necessary to perform this study in an animal model.

V.3.2. Animal Model and Species Justification: The Japanese quail was chosen for this study because it is a model species used in avian toxicity tests and it breeds readily in a laboratory setting. In addition, these birds only take approximately six-eight weeks to reach reproductive maturity, which greatly reduces the time required to produce multi-generations when compared to other similar species. The Northern Bobwhite, for example, takes an average of six months to reach reproductive maturity. A wide body of knowledge exists for Japanese quail.

V.3.3. Laboratory Animals

V.3.3.1. Genus species: *Coturnix japonica*

V.3.3.2. Strain / Stock / Breed: In-bred; nonspecific

V.3.3.3. Source / Vendor: GFQ Manufacturing Company Inc., Savannah, GA, or another vendor as coordinated through the AV

V.3.3.4. Age: Eggs-adult (~12-16 weeks)

V.3.3.5. Weight: Adult weight = ~100-300 g depending on strain

V.3.3.6. Sex: Males and females

V.3.3.7. Special Considerations: N/A

V.3.4. Number of Animals Required (by Species): 544

V.3.4.1. Avian Acute Oral Toxicity Test: As many as 54 birds could be needed for the Avian Acute Oral Toxicity Test, as many as 30 of which would be control animals, depending on observed mortalities.

V.3.4.2. F0 generation: Based on the hatch rate of 70% commonly observed at USAPHC with eggs obtained from Lake Cumberland Game Farm, a minimum of 60 eggs will be set to incubate per 5 treatment groups (potential for a maximum of 300 chicks with 100% hatchability or 210 with predicted hatchability). Using a 70% hatch rate is protective, as the hatch rate seen with eggs from other sources (i.e. University of Maryland) was as high as 90%.

In order for the F0 generation to proceed, 12 males and 12 females from each treatment group (60 males and 60 females, 120 total) will be kept for exposure to NTO. There will also be a group of 12 birds exposed at the highest concentration and then taken off dose to see if partial or full recovery of testicular mass occurs. An additional 4 birds (2 males and 2 females, if possible) will be kept as sentinels, and up to 8 additional birds (4 males and 4 females) per treatment level (40 total) may be used as potential replacements for birds unable to adapt to the automatic watering system upon transfer from the chick to adult caging. This makes for a total of 176 birds in the F0 generation, including sentinels and potential replacements. An additional 34 birds are expected to be culled as extras in the F0 generation.

V.3.4.3. F1 generation: Based on the hatch rate of 70% commonly observed at USAPHC, a minimum of 80 eggs will be set to incubate per 5 treatment groups (potential for a maximum of 400 chicks with 100% hatchability or 280 with predicted hatchability).

More eggs will be set for the F1 than the F0 to ensure appropriate sample size in the event that NTO exposure decreases fertility to as low as a 55% hatch rate. In order for the F1 generation to proceed, 12 males and 12 females from each treatment group (60 males and 60 females, 120 total) will be kept for exposure to NTO. An additional 8 birds (4 males and 4 females) per treatment level (40 total) may be used as potential replacements for birds unable to adapt to the automatic watering system upon transfer from the chick to adult caging. This makes for a total of 160 birds in the F1 generation including potential replacements. No sentinels are needed in the F1 generation, as they are carried over from the F0 generation; an additional 120 birds (extras in the F1 generation) are expected to be culled or transfered to a training protocol prior to transitioning birds to adult housing based on experience from previous studies.

V.3.5. Refinement, Reduction, Replacement (3 Rs):

V.3.5.1. Refinement: No additional refinements will be employed other than the environmental enrichment strategy.

V.3.5.2. Reduction: The one-generation study is designed to replace both the extended-one generation and two-generation reproductive toxicity studies, thereby reducing animal use. This study uses approximately 40% fewer animals than the two-generation study (OECD 2011). Additionally, this study will utilize groups of birds to determine reproductive, developmental, behavioral, and immunotoxicity effects in the same individuals. This allows a large reduction in the number of birds relative to the number that would be needed to measure these effects separately.

V.3.5.3. Replacement: No non-animal alternatives are known to exist that will provide the required data. At this time, there are no non-animal alternatives that can fully replicate the complex processes that occur within an intact organism.

V.4. Technical Methods:

V.4.1. Pain / Distress Assessment:

V.4.1.1. APHIS Form 7023 Information:

V.4.1.1.1. Number of Animals: 544

V.4.1.1.1.1. Column B: 4

V.4.1.1.1.2. Column C: 516

V.4.1.1.1.3. Column D: 0

V.4.1.1.1.4. Column E: 24

V.4.1.2. Pain Relief / Prevention

V.4.1.2.1. Anesthesia / Analgesia / Tranquilization: none

V.4.1.2.2. Pre- and Post-Procedural Provisions: A physical examination will be made at least once each day during all phases of the study. Observations will be detailed and carefully recorded in the study records. Details related to observations and/or physical examination of animals is described in sections V.1.1.3. and V.1.2.3.

The phlebotomy sites will be monitored for complications following blood collection. Based on experience, no complications following blood collection are anticipated. Appropriate fluid support will be provided by the veterinary and/or study staff based on signs indicative of dehydration or shock (e.g. lethargy, depression, wing droop, ruffled feathers, panting). For IV injections, a referenced safe maximum volume is 5 ml/kg body weight. For IP injections, a referenced safe maximum volume is 20 ml/kg.

V.4.1.2.3. Paralytics: N/A

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures:

V.4.1.3.1. Literature Source(s) Searched: FEDRIP, PubMed, Web of Science

V.4.1.3.2. Date of Search: 26 March 2014

V.4.1.3.3. Period of Search: 1900-2014

V.4.1.3.4. Key Words of Search: (3-nitro-1,2,4-triazol-5-one or 3 nitro 1,2,4 triazol 5 one or triazoles or nitro compounds) and ("endocrine disruption" or "endocrine disrupting chemical") and (japanese quail or corturnix japonica) and (pain or distress or refine or reduce or replace or artificial or vitro or culture or tissue or cell or organ or insect or arachnid or invertebrate or fish or mollusk or cephalopod or simulate or digital or interactive or mannequin or manikin or model)

V.4.1.3.5. Results of Search: The literature search identified 18 references pertaining to alternatives to painful procedures. However, no acceptable alternative to the painful or distressful situations potentially associated with the Avian Acute Oral Toxicity Test were found.

V.4.1.4. Unalleviated Painful or Distressful Procedure Justification: Quail may experience pain or distress associated with treatment in the Avian Acute Oral Toxicity Test. Mortality is the primary endpoint in this test, therefore, pain alleviating drugs cannot be administered. However, animals obviously in pain or showing signs of distress will be euthanized immediately (OECD 2009).

V.4.2. Prolonged Restraint and Restraint Methods: N/A

V.4.3. Surgery: N/A

V.4.3.1. Pre-surgical Provisions: N/A

V.4.3.2. Procedure: N/A

V.4.3.3. Post-surgical Provisions: N/A

V.4.3.4. Location: N/A

V.4.3.5. Surgeon: N/A

V.4.3.6. Multiple Survival Operative Procedures

V.4.3.6.1. Procedures: N/A

V.4.3.6.2 Scientific Justification: N/A

V.4.4. Animal Manipulations

V.4.4.1. Injections:

V.4.4.1.1. Foreign RBCs: Each individual will receive one 0.075-0.1 ml injection depending on animal weight of a 5-10% foreign RBC suspension in PBS. Injections will be performed IV or IP. For IV injections, a referenced safe maximum volume is 5 ml/kg body weight. For IP injections, a referenced safe maximum volume is 20 ml/kg. IV injections will be administered into the jugular vein found along the neck or at the brachial wing vein using a 1-3 ml syringe fitted with a 21-25 gauge needle or a tuberculin syringe. IP injections will be given in the caudal abdomen using a 1-3 ml syringe fitted with a 21-25 gauge needle or a tuberculin syringe. Immunotoxicity testing will be done after at least two weeks of treatment.

V.4.4.1.2. Fluid Support: Appropriate fluid support will be administered by the AV or his/her designees and/or study staff based on signs indicative of dehydration or shock described in V.4.1.2.2. Fluid injections may be administered IV or IP. Safe volumes for IV and IP injections are listed above and will be determined on a case by case basis by the AV or his/her designee. If fluids are given, either lactated ringers or 0.9% sodium chloride will be used. The AV or his/her designee may authorize the total volume given to exceed 5ml/kg IV or 20 ml/kg IP if medically necessary.

V.4.4.2. Use of Non-pharmaceutical-grade chemicals: The compound being tested (NTO) is not available in a pharmaceutical-grade composition. It is under investigation as described in the objective section of this protocol.

V.4.4.3. Biosamples: Blood will be collected approximately one week prior to and the day of euthanasia.

V.4.4.3.1. Blood Collection: Blood will be collected IAW VMD SOP 015.000. Blood will be collected from the jugular veins in unanesthetized quail. Quail will be restrained briefly while blood is collected; wings and legs will be kept firmly pressed while the injector brushes the feather away from the featherless tract in the neck region to expose the jugular vein. Water or alcohol may be used to part the feathers for better visualization of the vein.

A 1-3 ml syringe fitted with a 21-25-gauge needle will be inserted parallel with the vein. Tuberculin syringes or microcapillary tubes may also be used. Needle size will be optimized to bird size.

Optimally, the needle is to be inserted in the direction against blood flow, allowing the blood to flow towards the syringe which aids in clotting following the procedure. When sufficient volume is attained, gentle pressure will be applied peripheral to the venipuncture site, and maintained while the needle is withdrawn and afterwards until clotting is complete. This will minimize the formation of a hematoma. One milliliter of blood may be taken from non-terminal blood draws and up to 2 ml may be taken just prior to euthanasia.

V.4.4.4. Adjuvants: N/A

V.4.4.5. Monoclonal Antibody (MAb) Production: N/A

V.4.4.6. Animal Identification: Animals will be identified by cage cards according to VMD SOP 014.000 (USAPHC 2014d). Leg bands will be placed on the F0 generation immediately prior to assignment to treatment group. Leg bands will be placed on the F1 generation immediately after hatching. Legs will be observed regularly for tight bands that need to be replaced with larger leg bands. Animals will be identified by cage cards after birds are moved from chick to adult caging. Remaining birds will be euthanized within one week following the transfer. 390 birds will be identified by a cage card number.

V.4.4.7. Behavioral Studies: Initiation of normal male sexual behavior is important for successful reproduction. In reproductively mature males (when 90% of control males have reached reproductive maturity), trials shall be performed to assess reproductive behavior including: time to mount attempts, number of mount attempts, and number of successful cloacal contacts. Reproductively naïve males and females will be paired for approximately three minutes per each of three days for the copulatory behavior assessments. Pairing may last approximately 30 minutes; this time period has been shown to be effective in allowing mating to occur before aggressive effects are observed.

V.4.4.8. Other Procedures:

V.4.4.8.1. Oral Gavage: Birds are held firmly in one hand with their wings kept close to their bodies while the neck is outstretched. With beak open, the round tip gavage needle is inserted into the mouth and gently guided down the esophagus until making contact with the gizzard, at which point the contents of the syringe can be released. When the syringe is empty, the needle is gently withdrawn and the bird can be released back into its enclosure. NTO corn oil suspension will be tested prior to use to ensure that it can pass through the gavage needle.

V.4.4.8.2. Male Cloacal Gland: Growth of the male cloacal gland will be measured with a straight ruler on even weeks of age (2, 4, 6, etc.), and immediately prior to termination. Cloacal gland will also be measured on day of first foam.

V.4.4.9. Tissue Sharing: Tissues from animals euthanized on this study may be made available to other USAPHC personnel or other collaborating personnel if coordinated through the AV and SD/PI.

V.4.5. Study Endpoint: In the Avian Acute Oral Toxicity Test, mortality is the primary endpoint and background mortality is presumed to be negligible. During the test, animals obviously in pain or showing signs of severe distress will be euthanized. Dosed birds will be euthanized at the conclusion of the Avian Acute Oral Toxicity test (roughly

6 to 8 weeks of age). Birds that do not receive NTO (those that are held but not used due to the outcome of the acute testing stages) may be transferred to the training protocol after consultation with the AV or will be euthanized.

In the one generation study, birds from the F0 generation will be euthanized (as described in section V.4.6.) once all measures are completed and once eggs to produce the F1 generation are set to incubate (approximately 12 weeks of age). Birds from the F1 generation will be euthanized (as described in section V.4.6.) once behavioral assessment has been completed (at approximately 10 weeks of age).

Moribund or animals in overt pain will be humanely euthanized as described in section V.4.6. Based on previous experience with these models we will consider the following as indications of distress where recovery is unlikely at a continued exposure regime: loss of body weight (from the bird's last weight) to exceed 20%; loss of righting response; and inability to feed or drink without assistance.

A weight of evidence determination to euthanize will be made on a case-by-case basis for other animal welfare issues after consultation with the SD/PI and the AV. Potential replacements for each generation will be culled or transferred to the training protocol once the study birds demonstrate ability to drink successfully from the automatic watering system in the single housed conditions. The exact day of euthanasia or transfer to another protocol will be determined by the SD/PI following discussion with the AV.

V.4.6. Euthanasia: Birds will be euthanized via CO_2 as IAW VMD SOP 002.000. Death will be ensured by decapitation or cervical dislocation. Decapitation will occur with shears that will be kept sharp. Shears are sharpened as needed by the necropsy coordinator. The blades will be positioned to cut caudal to the base of the skull and cranial to the thoracic vertebrae. The blades will be closed using one swift smooth motion. When performing cervical dislocation, animals will be restrained with the abdomen facing down. Birds will be held in place at the base of the skull while the legs are simultaneously swiftly pulled back and slightly up.

Study staff and veterinary medicine personnel who are trained will perform euthanasia. If the SD/PI or other study staff is not available, the veterinary medicine staff will euthanize the animal and take as many required samples as possible from the animal, according to the protocol and upon training. Required samples include: heart, liver, spleen, thyroid, reproductive tissues (ovaries for females, testes and left and right epididymes for males), thymus, and bursa. The animal carcass will then be held at the facility under refrigeration for a minimum for three days in order for investigators to take any additional samples.

If not incubated, eggs will be broken prior to being discarded. At ED4, eggs that are not destined to hatch will be frozen (placed in an approximately 4ºC freezer for a minimum of four hours) or broken prior to being discarded. At ED17 and greater, embryos will be decapitated immediately following removal from the egg IAW VMD SOP 002.000.

V.5. Husbandry & Veterinary Care:

V.5.1. Husbandry Considerations: Birds will be randomly assigned to treatments within cages and cage units. Each cage will have individual stainless steel feeders and one stainless Edstrom cup drinker. Watering will be via the automatic watering system. Each cage dimension is 7" wide x 9.5" tall x 11" deep. Each cage will contain a cage card.

The photoperiod for chicks and adults will be 16 to 17 hours light and 8 to 7 hours of darkness. Chicks and juveniles will be group housed. Birds will be pair housed upon transition to adult caging (same sex; at approximately 4 weeks of age) and separated to single cages at approximately one week later, after demonstrating competent use of the watering system. From this point, birds will be single housed to maintain reproductive naivety for copulatory behavior testing. Even after copulatory behavior testing, birds will remain single housed, and thus will be mated via pairing that may last approximately 30 minutes; this time period has been shown to be effective in allowing mating to occur before aggressive effects are observed.

Hatchlings will be transferred to the chick enclosures the same day of hatch when completely dry. Once grouped, chicks will be checked at least twice daily to ensure that group shunning, hypothermia, and /or starvation do not occur. Chicks will be housed together by treatment group. At approximately four weeks, juveniles will be moved to adult cages and pair-housed (same sex). This will serve to ensure that juveniles learn to use the automatic watering system. Eight extra birds per treatment level may be brought over to the adult caging at this time to replace any birds that may not have learned how to use the automatic water troughs or any who need to be replaced because of aggression (toward another bird or injuries incurred from an aggressive pen mate). Once the birds demonstrate competent use of the water system, the pairs will be separated. This separation helps to aid in measuring onset of reproductive maturity (via foam and egg production) and reproductive behavior naivety (since males will likely mount each other if left pair-housed upon sexual maturity).

Table 5. Housing conditions

Age (weeks)	Temp. (ºC)	Temp. (ºF)	Relative Humidity (%)
Incubation	37.5-37.8	99.5-100	50-70
Hatch	37-37.5	98.6-99.5	70-75
1	35-38	95-100	30-70
2	30-35	86-95	30-70
3-4	23-30	74-86	30-70
> 4	21-27	70-80	30-70

Temperature inside the chick enclosures will be monitored by thermometers. Feed and water will be provided *ad libitum*. Chicks will receive a growth diet and adults will be given a laying diet that will accommodate the expenditure of calcium through egg laying.

Eggs may be stored in a cold storage facility (13-16 ºC) for up to two weeks prior to setting in an incubator. Cool storage prevents embryo development and aids in

synchronizing the development of embryos laid during the week. Alternatively, two to three days' worth of eggs may begin to be incubated every other day over one to two weeks to generate each generation. Reasons for this alternative method may include an inability to store eggs at optimum temperature/humidity and making hatch days more manageable with a reduction of the number of birds to be banded, weighed, dosed, etc. per day. All cracked and abnormal eggs will be removed prior to incubation and handled as described in section V.4.6. Eggs will be equilibrated to room temperature prior to being set in the incubator. Eggs should be turned in the incubator at least three times a day. Eggs can be transferred from the incubator to the hatcher one to three days prior to estimated day of hatch.

V.5.1.1. Study Room: This study will be conducted at the USAPHC TOX animal facility, Building E-2100 or E-2101, housing room as assigned. All live animal work will occur in the housing room. Bleeding and cloacal gland measuremnts directly prior to euthanasia will occur in the necropsy room, E-3201.

V.5.1.2. Special Husbandry Provisions: Veterinary medical personnel will administer feed and water. Water will be provided via the automated watering system and/or carboys. Chicks will be counted each day by veterinary medical personnel and the number of chicks in individual enclosures will be recorded. Veterinary medical personnel will set up the incubator, hatcher, and chick enclosures no less than one week prior to use in order to allow for time for temperature to acclimate. Temperature and relative humidity will be maintained by veterinary medical personal according to section V.5.1., checked twice daily, and documented. General husbandry procedures performed by the veterinary medical personnel will need to be performed with consideration of morning observations (i.e. foam checks, egg collection, copulatory behavior testing).

V.5.1.3. Exceptions: Once the birds demonstrate competent use of the water system, the pairs will be separated. This separation helps to aid in measuring onset of reproductive maturity (via foam and egg production) and reproductive behavior naivety (since males will likely mount each other if left pair-housed upon sexual maturity).

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care: Animals will routinely be observed no less than once daily by assigned veterinary medical personnel for husbandry conditions, humane care, and general health. In the event an animal becomes ill or injured, veterinary or toxicology personnel will immediately contact the AV or the designated backup who will determine the appropriate course of action. Animals will be assessed for moribundity based on: loss of body weight (from day 0) to exceed 20%; loss of righting response; an inability to feed or drink without assistance; and signs indicative of dehydration or shock (e.g. lethargy, depression, wing droop, ruffled feathers, panting). Animals considered to be moribund will immediately be euthanized as described in section V.4.6. The AV will be consulted to evaluate potentially moribund animals,

unless the SD/PI plans to immediately euthanize the animal. After exposure begins, animals will also be observed at least once daily by the SD/PI or study staff. Frequency of observations may be increased as needed following agreement between the AV and SD/PI.

V.5.2.2. Emergency Veterinary Medical Care: In the event an animal requires after-hours emergency veterinary care, a veterinarian is available 24 hours a day, 7 days a week. In the case of an emergency health problem, if the SD/PI or co-PI is unavailable or the investigator staff and veterinary staff cannot reach consensus on treatment of a study animal, the AV has the authority to treat the animal, remove it from the experiment, institute appropriate measures to relieve severe pain or distress, or perform euthanasia if necessary. However, all decisions involving the treatment of a study animal in which a consensus cannot be reached will only be made after the AV or designated backup veterinarian has actually observed and examined the animal in question. To facilitate communication, the animal care staff will maintain an emergency contact roster. In an emergency, the animal care staff will phone the numbers (office, home, and mobile) listed for the SD/PI and co-PI. If the SD/PI or co-PI cannot be reached by phone within 15 minutes, then they are considered unavailable.

V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy: Enrichment will consist of group housing hatchling and juveniles. After behavioral assessment, male and female quail will be paired regularly (Monday, Wednesday, and Friday*,* if possible). Food treats, stainless steel bells, natural fiber ropes (sisal), and other environmental enrichment items may be used and will be addressed in the enrichment plan. The enrichment plan will be posted outside of the animal room door when the study starts.

V.5.3.2. Enrichment Restrictions: N/A

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

Person	Activities	Training	Qualifications & Experience
Allison Jackovitz	Handling and observations	Chick and adult Japanese quail handling, health surveillance and observation (05/2012)	B.S., Biology 5+ years animal research
	Oral gavage	*TBS	
	Phlebotomy	Japanese quail jugular and brachial vein blood draws (08/2012)	
	Euthanasia	Prior to training documentation	
	Necropsy	Quail necropsy (11/2011)	
Terry Hanna	Handling and observations	Avian handling and blood withdraw techniques (01/2007)	ALAT (1992) 15+ years animal experience
	Oral gavage	*TBS	
	Phlebotomy	Avian handling and blood withdraw techniques (01/2007)	
	Euthanasia	Avian euthanasia (CO2) techniques in Japanese	

		quail (06/2009)	
	Necropsy	Japanese quail – necropsy (10/2009)	
Mike Quinn	Handling and observations	Avian handling and blood withdraw techniques (01/2007)	Ph.D., Animal Science 15+ years animal research
	Oral gavage	Avian handling and dosing in Bobwhite quail (03/2008)	
	Phlebotomy	Avian handling and blood withdraw techniques (01/2007)	
	Euthanasia	Avian euthanasia (CO2) techniques in Japanese quail (06/2009)	
	Necropsy	Necropsy – quail (06/2005)	
Lee Crouse	Handling and observations	Prior to training documentation	M.S., Environmental Science 16+ years animal research
	Oral gavage	Prior to training documentation	
	Phlebotomy	Prior to training documentation	
	Euthanasia	Prior to training documentation	
	Necropsy	Prior to training documentation	
Adam Deck	Handling and observations	*TBS	B.S., Biology 12+ years animal experience
	Oral gavage	*TBS	
	Phlebotomy	*TBS	
	Euthanasia	*TBS	
	Necropsy	*TBS	
Rachel Hebert	Handling and observations	*TBS	B.S., Biology 3+ years animal research
	Oral gavage	*TBS	
	Phlebotomy	*TBS	
	Euthanasia	*TBS	
	Necropsy	*TBS	
Emily Lent	Handling and observations	Avian handling and health surveillance (01/2009)	Ph.D., Natural Resources and Environmental Studies 13+ years animal research
	Oral gavage	Avian handling and dosing in Bobwhite quail (03/2008)	
	Phlebotomy	N/A	
	Euthanasia	Japanese quail – CO2 euthanasia (10/2009)	
	Necropsy	Japanese quail – necropsy (10/2009)	
Emily Reinke	Handling and observations	Chick and adult Japanese quail handling, health surveillance and observation (06/2012)	Ph.D., Pathology 5 years animal experience
	Oral gavage	*TBS	
	Phlebotomy	N/A	
	Euthanasia	*TBS	
	Necropsy	N/A	
Mark Way	Handling and observations	Bird handling and dosing (12/2007)	B.S., Biology 20+ years animal research
	Oral gavage	Bird handling and dosing (12/2007)	
	Phlebotomy	Avian bleeding – jugular (06/2009)	
	Euthanasia	CO2 euthanasia for Japanese quail (10/2009)	

	Necropsy	Necropsy of Japanese quail (10/2009)	

VII. BIOHAZARD/SAFETY: Risks associated with this protocol include bites/scratches/needle sticks, transmission of zoonotic diseases, and the development of animal allergies. To minimize risk, appropriate handling techniques will be used and appropriate PPE will be worn for all animal handling work. This includes (but may not be limited to) facemask, gloves, and disposable lab coat. Personnel will wash their hands upon completion of animal work. Applicable current TOX SOPs and PHC regulations (TOX SOP 046.002 and USACHPPM 385-5, OHS of Animal Users) will be followed. These documents specify hazardous waste disposal, bite/scratch procedures, and zoonotic disease prevention. A sharps container will be present at all times when using sharps and needles will not be recapped after entering animal tissue.

Birds have been reported to carry specific zoonotic diseases that can be transferred to humans (i.e. psittacosis, histoplasmosis), most of them affecting the respiratory system; however, since transmission of this sort usually occurs through dry fecal material in wild birds, the likelihood of this hazard in the lab setting from laboratory-raised birds is relatively low. Adherence to standard chemical and animal handle procedures will be required during the performance of this study. Additionally, all personnel participating in this study will act according to USACHPPM 385-5 and use proper PPE, including dust masks or respirators, according to TOX SOP 046.002.

VIII. ENCLOSURES: N/A

Animal Use Protocol: One-generation reproductive toxicity test in Japanese quail *(Corturnix japonica)* using 3-nitro-1,2,4-triazol-5-one (NTO)

IX. ASSURANCES:

IX.1. As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken into consideration and made the proper coordination regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures / manipulations / observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures / manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely, "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL / WILL NOT (circle one or both, if applicable) be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

I. Unexpected Adverse Events: I acknowledge the responsibility for reporting unexpected adverse events IAW the most current version of IACUC Policy Memorandum No. 8. "Policy on Unexpected Adverse Event Reporting".

Allison M. Jackovitz	AJackovitz	3 July 14
(PRINT) Principal Investigator	(Signature)	(Date)

IX.2. As the Primary Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Authority: I understand that, as the Primary Co-Investigator, I am authorized and responsible for performing all procedures and manipulations as assigned to the SD/PI in the SD/PI's absence. This includes euthanasia of distressed animals.

C. Training: I verify that I am technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

D. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that I will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

E. Painful Procedures: I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL or WILL NOT (circle one or both, if applicable) be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

F. Unexpected Adverse Events: I acknowledge the responsibility for reporting unexpected adverse events IAW the most current version of IACUC Policy Memorandum No. 8. "Policy on Unexpected Adverse Event Reporting".

Theresa L Hanna

(PRINT) First name, MI, Last name of Primary Co-Investigator

Theresa L Hanna 7/3/14

(Signature) (Date)

IX.3. As a Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Authority: I understand that, as a Co-Investigator, I am authorized, responsible for, and willing to perform all procedures and manipulations as assigned to me by the SD/PI.

C. Training: I verify that I am technically competent and have been or will be properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the assigned procedures/manipulations performed by me.

D. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that I will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to participate in this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

E. Painful Procedures: I am participating in biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. I will follow the direction of the SD/PI relative to potential pain and/or distress and relief by use of anesthetics, analgesics, and/or tranquilizers.

F. Unexpected Adverse Events: I acknowledge the responsibility for reporting unexpected adverse events IAW the most current version of IACUC Policy Memorandum No. 8. "Policy on Unexpected Adverse Event Reporting".

Michael J. Quinn — 3 Jul 2014

(PRINT) First name, MI, Last name of Co-Investigator | (SIGNATURE) | (DATE)

(PRINT) First name, MI, Last name of Co-Investigator | (SIGNATURE) | (DATE)

(PRINT) First name, MI, Last name of Co-Investigator | (SIGNATURE) | (DATE)

(PRINT) First name, MI, Last name of Co-Investigator | (SIGNATURE) | (DATE)

APPENDIX A

REFERENCES

Crouse, L.C.B., et al. 2010. Subchronic Oral Toxicity of NTO in Rats. U.S. Army Public Health Command, Aberdeen Proving Ground, MD. Toxicology Study No. 85-XC-0A6W-08.

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USAPHC. 2014b. VMD SOP 007.000, Animal Quality Assurance and Quality Control/Health Monitoring Procedures. Aberdeen Proving Ground, Maryland.

USAPHC. 2014c VMD SOP 015.000, Animal Bleeding Techniques. Aberdeen ProvingGround, Maryland.

USAPHC. 2014d. VMD SOP 014.000, Individual Animal Identification. Aberdeen Proving Ground, Maryland.

USAPHC. 2014e. VMD SOP 002.000, Animal Euthanasia. Aberdeen Proving Ground, Maryland.

USAPHC. 2014f. TOX SOP 046.002, Health and Safety of Laboratory Personnel, Aberdeen Proving Ground, Maryland.

PROTOCOL REVIEW, SUPPORT, APPROVAL SHEET

PROTOCOL NUMBER: - 80 - 14-07-02 (SUB-JONO / TEST TYPE / IACUC NUMBER)	TITLE: One-generation reproductive toxicity test in Japanese quail *(Coturnix japonica)* using 3-nitro-1,2,4-triazol-5-one (NTO)

1. SCIENTIFIC MERIT (PEER REVIEW)			
1a. Printed Name (First, MI, Last) Matthew, A, Bazar	1b. Title Biologist	1c. Signature BAZAR.MATTHEW.A.1241429322	1d. Date (yyyy/mm/dd) 20140407
2. DIRECTOR			
2a. Printed Name (First, MI, Last)	2b. Title	2c. Signature Click to Approve	2d. Date (yyyy/mm/dd)
3. PROGRAM MANAGER			
3a. Printed Name (First, MI, Last) Michael, J, Quinn, Jr.	3b. Title Health Effects Research Program Manager	3c. Signature QUINN.MICHAEL.J.JR.1282372092	3d. Date (yyyy/mm/dd) 20140407
4. ATTENDING VETERINARIAN			
4a. Printed Name (First, MI, Last) Dawn, C, Fitzhugh	4b. Title Attending Veterinarian	4c. Signature FITZHUGH.DAWN.CATHERINE.1036926127	4d. Date (yyyy/mm/dd) 20140407
5. ANALYTICAL CHEMISTRY (If Applicable)			
5a. Printed Name (First, MI, Last)	5b. Title	5c. Signature Click to Approve	5d. Date (yyyy/mm/dd)
6. SAFETY MANAGER			
6a. Printed Name (First, MI, Last)	6b. Title	6c. Signature Click to Approve	6d. Date (yyyy/mm/dd)
7. STATISTICIAN (If Applicable)			
7a. Printed Name (First, MI, Last) Karen, D, Deaver	7b. Title Senior Command Statistician	7c. Signature DEAVER.KAREN.DEVILBISS.1400519672	7d. Date (yyyy/mm/dd) 20140410

PROTOCOL NUMBER: - - 14-07-02 (SUB-JONO / TEST TYPE / IACUC NUMBER)	TITLE: One-generation reproductive toxicity test in Japanese quail (Coturnix japonica) using 3-nitro-1,2,4-triazol-5-one (NTO)		
8. SIO-QAT (GLP COMPLIANCE AND QA SUPPORT)			
8a. Printed Name (First, MI, Last)	8b. Title	8c. Signature	8d. Date (yyyy/mm/dd)
		Click to Approve	
9. CHAIRMAN, IACUC			
9a. Printed Name (First, MI, Last)	9b. Title	9c. Signature	9d. Date (yyyy/mm/dd)
Kristin T. Newkirk	Animal Care and Use Specialist	NEWKIRK.KRISTIN.TORELL.1014786895	20140703
10. INSTITUTIONAL OFFICIAL			
10a. Printed Name (First, MI, Last)	10b. Title	10c. Signature	10d. Date (yyyy/mm/dd)
John J. Resta	Director, Army Institute of Public Health	RESTA.JOHN.J.1229129305	20140710
11. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR			
11a. Printed Name (First, MI, Last)	11b. Title	11c. Signature	11d. Date (yyyy/mm/dd)
Allison, M, Jackovitz	Biologist	JACKOVITZ.ALLISON.M.1367161236	20140417
12. OTHER ORGANIZATION(S) PROVIDING SUPPORT (AS NEEDED):			
12a. Printed Name (First, MI, Last)	12b. Title	12c. Signature	12d. Date (yyyy/mm/dd)
13. STUDY SPONSOR:			
13a. Printed Name (First, MI, Last)	13b. Title	13c. Signature	13d. Date (yyyy/mm/dd)

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2014/12/18	2. PROTOCOL NUMBER: 80-14-07-02	3. MODIFICATION#: 1

4. PROTOCOL TITLE: One-generation reproductive toxicity test in Japanese quail (Corturnix japonica) using 3-nitro-1,2,4-triazol-5-one (NTO)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Allison M. Jackovitz	410-436-8772	PHC/TOX/TEP

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS:

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX)

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY:					1b. N/A ☑				
2. ORIGINAL PROTOCOL TOTAL: 544					3. PROTOCOL TOTAL AFTER MODIFICATION: 544				
2a. USDA pain cat:	B: 4	C: 516	D: 0	E: 24	3a. USDA pain cat:	B: 4	C: 516	D: 0	E: 24

4. Yes	No	
☐	☑	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☑	☐	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☑	☐	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

PROTOCOL Page, paragraph, section	SECTION III. MODIFICATION/JUSTIFICATION *Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals*
Page 19 Section V.5.1 Table 5	1. MODIFICATION: Incubator should be between 99.0 and 100.0 degrees Farenheit.
	1a. JUSTIFICATION/REASON: Temperature range originally specified in protocol is too narrow.

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
Page 21 Section VI.	2. MODIFICATION: Addition of Matthew Bazar, Marc Williams, and Stephen Rice to study staff. Removal of Rachel Hebert. See attached Vi. Study Personnel Qualifications and Training. (SEE ATTACHED TABLE)
	2a. JUSTIFICATION/REASON: Additional study staff are necessary as the study involves daily dosing of Japanese quail. A robust study staff is needed in order to administer the compound daily (including weekends and holidays).
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES [X] NO [✓]

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Allison Jackovitz	Signature a Jackovitz	DATE: (yyyy/mm/dd) 2014/12/18
2. PROGRAM MANAGER:: (Printed Name) ARTHUR J. O'NEILL	Signature Arthur J. O'Neill	DATE: (yyyy/mm/dd) 2014/12/19
3. ATTENDING VETERINARIAN: (Printed Name) MAJ Mary E. Sprangel VC, Veterinarian	Signature Mary E Sprangel	DATE: (yyyy/mm/dd) 2014/12/18
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE)	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) KRISTIN NEWKIRK	APPROVED / REVIEWED YES [✓] NO [ ] Signature Kristin Newkirk	DATE: (yyyy/mm/dd) 2014/12/19

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

Person	Activities	Training	Qualifications & Experience
Allison Jackovitz	Handling and observations	Chick and adult Japanese quail handling, health surveillance and observation (05/2012)	B.S., Biology 5+ years animal research
	Oral gavage	*TBS	
	Phlebotomy	Japanese quail jugular and brachial vein blood draws (08/2012)	
	Euthanasia	Prior to training documentation	
	Necropsy	Quail necropsy (11/2011)	
Terry Hanna	Handling and observations	Avian handling and blood withdraw techniques (01/2007)	ALAT (1992) 15+ years animal experience
	Oral gavage	*TBS	
	Phlebotomy	Avian handling and blood withdraw techniques (01/2007)	
	Euthanasia	Avian euthanasia (CO2) techniques in Japanese quail (06/2009)	
	Necropsy	Japanese quail – necropsy (10/2009)	
Mike Quinn	Handling and observations	Avian handling and blood withdraw techniques (01/2007)	Ph.D., Animal Science 15+ years animal research
	Oral gavage	Avian handling and dosing in Bobwhite quail (03/2008)	
	Phlebotomy	Avian handling and blood withdraw techniques (01/2007)	
	Euthanasia	Avian euthanasia (CO2) techniques in Japanese quail (06/2009)	
	Necropsy	Necropsy – quail (06/2005)	
Lee Crouse	Handling and observations	Prior to training documentation	M.S., Environmental Science 16+ years animal research
	Oral gavage	Prior to training documentation	
	Phlebotomy	Prior to training documentation	
	Euthanasia	Prior to training documentation	
	Necropsy	Prior to training documentation	
Adam Deck	Handling and observations	*TBS	B.S., Biology 12+ years animal experience
	Oral gavage	*TBS	
	Phlebotomy	N/A	
	Euthanasia	*TBS	
	Necropsy	N/A	
Emily Lent	Handling and observations	Avian handling and health surveillance (01/2009)	Ph.D., Natural Resources and Environmental Studies 13+ years animal research
	Oral gavage	Avian handling and dosing in Bobwhite quail (03/2008)	
	Phlebotomy	N/A	
	Euthanasia	Japanese quail – CO2 euthanasia (10/2009)	
	Necropsy	Japanese quail – necropsy (10/2009)	
Emily Reinke	Handling and observations	Chick and adult Japanese quail handling, health surveillance and observation (06/2012)	Ph.D., Pathology

	Oral gavage	*TBS	5 years animal experience
	Phlebotomy	N/A	
	Euthanasia	*TBS	
	Necropsy	N/A	
Mark Way	Handling and observations	Bird handling and dosing (12/2007)	B.S., Biology 20+ years animal research
	Oral gavage	Bird handling and dosing (12/2007)	
	Phlebotomy	Avian bleeding – jugular (06/2009)	
	Euthanasia	CO2 euthanasia for Japanese quail (10/2009)	
	Necropsy	Necropsy of Japanese quail (10/2009)	
Matt Bazar	Handling and observations	*TBS	M.S., Biology 13+ years research
	Oral gavage	*TBS	
	Phlebotomy	N/A	
	Euthanasia	N/A	
	Necropsy	N/A	
Marc Williams	Handling and observations	*TBS	Ph.D., Immunology and Hematology 25+ years animal research
	Oral gavage	*TBS	
	Phlebotomy	N/A	
	Euthanasia	*TBS	
	Necropsy	N/A	
Stephen Rice	Handling and observations	*TBS	B.S., Biology 6 months animal experience
	Oral gavage	*TBS	
	Phlebotomy	N/A	
	Euthanasia	*TBS	
	Necropsy	N/A	

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2015/03/04	2. PROTOCOL NUMBER: 80-14-07-02	3. MODIFICATION#: 2

4. PROTOCOL TITLE: One-generation reproductive toxicity test in Japanese quail (Coturnix japonica) using 3-nitro-1,2,4-tirazol-5-one (NTO)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Allison M. Jackovitz	410-436-8772	PHC/TOX/TEP

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS:

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX)
1	Incubator should be between 99.0 and 100.0 degrees Fahrenheit.	N/A	18 Dec 2014
1	Addition of Matthew Bazar, Marc Williams, and Stephen Rice. Removal of Rachel Hebert.	N/A	18 Dec 2014
GLP-1	Cage pads will be changed no less than every two days.	N/A	26 Feb 2015

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY:					1b. N/A ☑				
2. ORIGINAL PROTOCOL TOTAL: 544					3. PROTOCOL TOTAL AFTER MODIFICATION: 584				
2a. USDA pain cat:	B: 4	C: 516	D: 0	E. 24	3a. USDA pain cat:	B: 6	C: 554	D: 0	E: 24

4. Yes	No	
☑	☐	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☑	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☑	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

PROTOCOL Page, paragraph, section	SECTION III. MODIFICATION/JUSTIFICATION *Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals*
V. Materials and Methods, page 6	1. MODIFICATION: V.1. Experimental Design and General Procedures: Table 2. (see attached) Of the 260 quail hatched for the F0 generation, 254 will be dosed between weeks 2 and 4. All 254 treatment birds will be identified with a leg band. The other 6 will serve as sentinels and will not be banded. At week 4, the number of quail will be reduced to 160 and quail will be transferred to adult housing. These 160 quail and will continue receiving NTO via oral gavage. All 160 quail will be identified with a cage card. At week 5, the number of quail will be reduced to 120. These 120 quail and will continue receiving NTO via oral gavage. Each time number of quail is reduced, control birds (i.e. those who do not receive NTO) may be adopted. All birds other birds will be euthanized.
	1a. JUSTIFICATION/REASON: Due to an unexpectedly high hatch rate, we ended up with 260 birds rather than the anticipated 210. The high hatch rate has been taken into account for the setting of F1 eggs. As we cannot determine the sex of quail until 4-6 weeks of age, 254 birds will be spread across the five dose groups to ensure 12 mating pairs per dose group and 12 extra high dose birds (recovery birds). Twelve mating pairs per dose group and 12 recovery birds are necessary for appropriate statistical analysis. Previous studies have shown that some birds are unable to adapt to the automatic watering system upon their transition from the chick housing to adult caging at approximately 4 weeks of age. Although this inability to learn how to use the automatic watering system is minimized by moving the birds to the new caging as pairs, birds that are unable to make the transition will be replaced with those who can so that an adequate number of birds can achieve reproductive maturity in order to produce the subsequent generation.

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
V. Materials and Methods, page 8	2. MODIFICATION: V.1.2. One-generation study: As many as 10 quail will be kept as sentinels, per VMD SOP 007.000, due to limited vendor history and health status of animals. Sentinels will be maintained through the conclusion of the study or euthanized and necropsied periodically to assess the health of quail in the room. Sentinels not euthanized and necropsied as part of the sentinel program can be adopted.
	2a. JUSTIFICATION/REASON: Sentinels are considered for studies lasting more than 90 days.
V. Materials and Methods, page 14	3. MODIFICATION: V.3.4.3. F1 generation: No sentinels are needed in the F1 generation, as they are carried over from the F0 generation. However, up to 4 sentinels MAY be added for the F1 generation. These sentinels will be generated from untreated parents. Sentinels not euthanized and necropsied as part of the sentinel program can be adopted.
	3a. JUSTIFICATION/REASON: In consult with the Attending Veterinarian, SD may add up to 4 additional sentinels when F1 chicks are moved to adult caging (for a total of 10 throughout the study). These 4 additional sentinels will be maintained through the conclusion of the study or euthanized and necropsied periodically to assess the health of quail in the room.
V. Materials and Methods, page 11	4. MODIFICATION: V.1.2.8 Immunotoxicity Testing: Blood will be collected (as described in section V.4.4.3.) prior to the injection (at baseline) and not to exceed 10 days after the injection to measure antibody response.
	4a. JUSTIFICATION/REASON: Window currently specified in protocol is too narrow.

Continued on next page YES ☑ NO ☐

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Allison M Jackovitz	Signature aJackovitz	DATE: (yyyy/mm/dd) 20150306
2. PROGRAM MANAGER:: (Printed Name)	Signature	DATE: (yyyy/mm/dd)
3. ATTENDING VETERINARIAN: (Printed Name) MARY E. SPRANGEL	Signature May E Sprangel	DATE: (yyyy/mm/dd) 20150307
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE)	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) KRISTIN NEWKIRK	APPROVED / REVIEWED YES ☑ NO ☐ Signature	DATE: (yyyy/mm/dd) 20150309

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
V.4.1.1.1-4, Number of animals per pain category, page 14	5 MODIFICATION: V.4.1.1.1.1. Column B = 6 (reflects sentinels from F0 birds) V.4.1.1.1.2. Column C = 554 (reflects 30 from acute study + 254 from the F0 generation + up to 270 from the F1 generation) V.4.1.1.1.3. Column D = 0 V.4.1.1.1.4. Column E = 24 (reflects those estimated as E for the acute study)
	5a JUSTIFICATION/REASON: The new pain category estimations take into account the number of birds actually hatched for the F0 generation and those anticipated to hatch in the F1 generation. The actual pain category usage will be reported for all birds at the end of the study.
V. Materials and Methods, page 9	6 MODIFICATION: Addition of control recovery group. Protocol already specifies that a group of 12 birds from the F0 generation will be exposed at the highest concentration and then taken off treatment to see if partial or full recovery of testicular mass occurs. A control group of 12 birds will be included from the F0 birds. The dosed and control recovery birds will be maintained through the duration of the F1 generation.
	6a JUSTIFICATION/REASON: A control group is needed to compare to the high dose recovery group treatment group.
	MODIFICATION:
	JUSTIFICATION/REASON:
	MODIFICATION:
	JUSTIFICATION/REASON:

Modification #2 attachment **Submitted: 03 March 2015**

Protocol: 80-14-07-02, "One-generation reproductive toxicity test in Japanese quail (*Coturnix japonica*) using NTO"

Table 2. Number of eggs to be set and quail to be used in the One-Generation Study
(The number of quail used in the previously conducted acute oral toxicity study is not included.)

One-Generation Study			
Group	**No. Quail**		**Pain Category**
F0 eggs set	300 Eggs		
F0 chicks produced	260		
Weeks 2-4: 254 quail dosed	254		254 C
Weeks 2+: *6 sentinels designated (quail are not dosed)*	***6 (either sex)***		6 B
	Males	**Females**	
Weeks 4-5: *160 quail selected for continued dosing*	**16 x 5 dose groups = 80*	**16 x 5 dose groups = 80*	
Weeks 5+: *120 quail selected for continued dosing***	*12 x 5 dose groups = **60***	*13 x 5 dose groups = **60***	
Weeks 2+: *24 recovery birds (12 NTO high/ 12 control dosed through F0 period. All are maintained through F1.)*	***24***	N/A	
Totals= *Note Italics indicates birds identified by cage card*	****150* **birds** of 260 produced will be used on study or as sentinels		**254 C, 6 B**
Estimated F1 eggs set 5 Groups; *60 eggs each*	5 x 60 = 300 Eggs		
Estimated F1 chicks produced ~70-90% hatch rate 300 eggs x 0.7 = 210 300 eggs x 0.9 = 270	210-270		
Day 2–week 4: 210-270 quail dosed	210-270		210-270 C
Day 2+: *0-4 sentinels designated (quail are not dosed)*	***0-4 (either sex)***		0-4 B
	Males	**Females**	
Weeks 4-5: 160 quail selected for continued dosing	**16 x 5 dose groups = 80*	**16 x 5 dose groups = 80*	
*Weeks 5+: 120 quail selected for continued dosing***	*12 x 5 dose groups = **60***	*13 x 5 dose groups = **60***	
Totals= *Note Italics indicates birds identified by cage card*	****120-124* birds of 210-270 produced will be used on study or as sentinels		**210-270 C, 0-4 B**
	One-Generation Study TOTAL		**464-524 C, 6-10 B**

* The number of males and females listed in the F0 and F1 group at the 4-5 weeks groups is estimated to be 80 males and 80 females. Sex determination is made during that time and at the actual numbers of each sex may vary but will be reported to the IACUC with the end of study animal usage information.

** Birds will be used through the duration of the dosing period.

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2015/03/19	2. PROTOCOL NUMBER: 80-14-07-02	3. MODIFICATION#: 3

4. PROTOCOL TITLE: One-generation reproductive toxicity test in Japanese quail (Coturnix japonica) using 3-nitro-1,22,4-triazol-5-one (NTO)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Allison M. Jackovitz	410-436-8772	PHC/TOX/TEP

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS:

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX)
1	Incubator should be between 99.0 and 100.0 degrees Farenheit. Addition/removal of study personnel.	N/A	18 Dec 2015
GLP-1	Cage pads will be changed no less than every two days.	N/A	26 Feb 2015
2	Amendment of Table 2 due to unepectedly high hatch rate. Increase in the number of sentinels. Addition of F1 sentinels (possibly).	N/A	3 Mar 2015
2	Wider blood draw window for RBC challenge. Adendment of Pain Categories. Addition of control recovery group.	N/A	3 Mar 2015

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 1b. N/A ☑

2. ORIGINAL PROTOCOL TOTAL: 544					3. PROTOCOL TOTAL AFTER MODIFICATION: 584				
2a. USDA pain cat:	B: 4	C: 516	D: 0	E. 24	3a. USDA pain cat:	B: 6	C: 554	D: 0	E: 24

4. Yes	No	
☑	☐	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☑	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☑	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

PROTOCOL Page, paragraph, section	SECTION III. MODIFICATION/JUSTIFICATION *Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals*
V. Materials and Methods, page 9	1. MODIFICATION: Addition of up to 12 recovery birds to the medium high (500 mg/kg) dose group. Protocol already specifies that two groups of 12 male birds from the F0 generation will be exposed at the control (0) and high (1000 mg/kg) doses through the dosing period of the F0 birds (~12 weeks), and then taken off treatment to see if partial or full recovery of testicular mass occurs. A group of up to 12 birds from the medium high dose F0 birds will be included as another recovery group. The high, medium high, and control recovery birds will be maintained through the duration of the F1 generation. No additional animals are being requested. The requested animals (up to 12) are "extra" birds from the 500 mg/kg dose group (medium high) which are already being dosed (dosing began at 2 weeks of age). The experimental design has all the chicks receiving daily gavage beginning at 2 weeks old, and continuing until it is determined which birds are selected for the F0 study (determined at approx. 5 weeks of age when they are sexed and moved into adult caging). The protocol specifies that "extra" birds that are not selected for study are culled (euthanized), or transferred to the training protocol (control birds only). The birds used for this additional recovery group are those that would otherwise be culled (euthanized). 1a. JUSTIFICATION/REASON: After 12 days of dosing, NTO appears to be more toxic to birds than anticipated. A 500 mg/kg (medium high) recovery group is needed in the event that the high dose group birds are unable to complete/survive the 12 weeks of dosing. After repeated doses, one bird has been found dead and many others in the 1000 mg/kg dose group have been euthanized early in accordance with the protocol early endpoints. By utilizing some of the "extra" male birds from the 500 mg/kg dose group as a recovery group rather than euthanizing them upon transfer to adult cages, we better ensure having recovery data which is an important component in this study.

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
	2. MODIFICATION:
	2a. JUSTIFICATION/REASON:
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☑

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Allison Jackovitz	Signature: A Jackovitz	DATE: (yyyy/mm/dd) 20150326
2. PROGRAM MANAGER:: (Printed Name)	Signature	DATE: (yyyy/mm/dd)
3. ATTENDING VETERINARIAN: (Printed Name) MAJ Mary E. Sprangel	Signature: MAJ Mary E. Sprangel	DATE: (yyyy/mm/dd) 20150326
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE)	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) Kristin T. Newkirk	APPROVED / REVIEWED YES ☑ NO ☐ Signature: Kristin T. Newkirk	DATE: (yyyy/mm/dd) 20150326

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2015/05/01	2. PROTOCOL NUMBER: 80-14-07-02	3. MODIFICATION#: 4

4. PROTOCOL TITLE: One-generation reproductive toxicity test in Japanese quail (Coturnix japonica) using 3-nitro-1,2,4-triazol-5-one (NTO)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Allison M. Jackovitz	410-436-8772	PHC/TOX/TEP

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS:

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX)
1	Incubator should be between 99.0 and 100.0 degrees Farenheit. Addition/removal of study personnel.	N/A	18 Dec 2015
GLP-1	Cage pads will be changed no less than every two days.	N/A	26 Feb 2015
2	Amendment of Table 2 due to unepectedly high hatch rate. Increase in the number of sentinels. Addition of F1 sentinels (possibly).	N/A	3 Mar 2015
2	Wider blood draw window for RBC challenge. Adendment of Pain Categories. Addition of control recovery group.	N/A	3 Mar 2015
3	Addition of 12 recovery birds to the medium high (500 mg/kg) dose group.	N/A	26 Mar 2015

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY:					1b. N/A ☑				
2. ORIGINAL PROTOCOL TOTAL:					3. PROTOCOL TOTAL AFTER MODIFICATION:				
2a. USDA pain cat:	B: 4	C: 516	D: 0	E: 24	3a. USDA pain cat:	B: 6	C: 554	D: 0	E: 24

4. Yes	No	
☐	☑	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☑	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☑	☐	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

PROTOCOL Page, paragraph, section	SECTION III. MODIFICATION/JUSTIFICATION *Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals*
Page 21 Section VI.	1. MODIFICATION: Removal of Matthew Bazar, Marc Williams, and Emily Reinke from Study Staff.
	1a. JUSTIFICATION/REASON: Due to fewer dose groups, and thus animals, workload is reduced. Personnel are no longer needed.

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
	2. MODIFICATION:
	2a. JUSTIFICATION/REASON:
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☑

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Allison Jackovitz	Signature aJackovitz	DATE: (yyyy/mm/dd) 2015/05/06
2. PROGRAM MANAGER:: (Printed Name)	Signature	DATE: (yyyy/mm/dd)
3. ATTENDING VETERINARIAN: (Printed Name) Mary Sprangel	Signature MVSprangel	DATE: (yyyy/mm/dd) 2015/05/26
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE)	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) KRISTIN NEWKIRK	APPROVED / REVIEWED YES ☒ NO ☐ Signature	DATE: (yyyy/mm/dd) 2015/05/28

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2015/05/01	2. PROTOCOL NUMBER: 80-14-07-02	3. MODIFICATION#: 5

4. PROTOCOL TITLE: One-generation reproductive toxicity test in Japanese quail (Coturnix japonica) using 3-nitro-1,2,4-triazol-5-one (NTO)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Allison M Jackovitz	410-436-8772	PHC/TOX/TEP

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS:

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX)
1	Incubator should be between 99.0 and 100.0 degrees Farenheit. Addition/removal of study personnel.	N/A	18 Dec 2015
GLP-1	Cage pads will be changed no less than every two days.	N/A	26 Feb 2015
2	Amendment of Table 2; Increase in sentinels; Wider blood draw window for RBC challenge; Adendment of Pain Categories; Addition of control recovery.	N/A	3 Mar 2015
3	Addition of 12 recovery birds to the medium high (500 mg/kg) dose group.	N/A	26 Mar 2015
4	Removal of study personnel	N/A	28 May 2015

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY:					1b. N/A ✓				
2. ORIGINAL PROTOCOL TOTAL:					3. PROTOCOL TOTAL AFTER MODIFICATION:				
2a. USDA pain cat.	B: 4	C: 516	D: 0	E: 24	3a. USDA pain cat:	B: 6	C: 554	D: 0	E: 24

4. Yes	No	
	✓	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.1. of the template.)
	✓	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
✓		Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

SECTION III. MODIFICATION/JUSTIFICATION

Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals

PROTOCOL Page, paragraph, section	
V.1.2 One -generation study	1. MODIFICATION: Early euthanasia of control recovery animals. Protocol currently states recovery males from the F0 generation will be euthanized (at approximately 23 weeks of age) when the F1 animals are euthanized
	1a. JUSTIFICATION/REASON: Since both the high and medium-high recovery birds have already died or been euthanized, there are no treatment animals to which the controls can be compared.

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
	2. MODIFICATION:
	2a. JUSTIFICATION/REASON:
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES NO ✓

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Allison Jackovitz	Signature	DATE: (yyyy/mm/dd) 20150610
2. PROGRAM MANAGER:: (Printed Name)	Signature	DATE: (yyyy/mm/dd)
3. ATTENDING VETERINARIAN: (Printed Name) Kenneth E. Despain	Signature	DATE: (yyyy/mm/dd) 20150610
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE)	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) KRISTIN NEWKIRK	APPROVED / REVIEWED YES NO Signature	DATE: (yyyy/mm/dd) 20150610

USAPHC IACUC Protocol Modification Review/Approval

Study Director/ Principal Investigator: **Allison Jackovitz**

PROTOCOL #: **80-14-07-02** Protocol Approval Date: 03 July 2014

PROTOCOL TITLE: **One-generation reproductive toxicity test in Japanese quail (Coturnix japonica) using 3-nitro-1,2,4-triazol-5-one (NTO)**

Phone No.: 410-436-8772 FAX No.:

Email: allison.m.jackovitz.civ@mail.mil

Modification Request #: 6 Submission Date: 2 July 2015

7/2/2015

X AJackovitz

Study Director/ Principal Investigator
Signed by: JACKOVITZ.ALLISON.M.1367161236

For Animal Use Review Office use only: Received Date: 2 July 2015

Review Process: VVC ☐ FCR ☐ DMR ☒

Member(s) initials: KN

IACUC Chair Review:

KRISTIN NEWKIRK 7 July 2015

Print Signature Date

Attending/Alternate Veterinarian Review: **Approved via VVC**: N/A ☐ YES ☐ NO ☐

Kenneth E. Despain 7 July 2015

Print Signature VVC Approval Date

SEMO Review *(if applicable)*:

Print Signature Date

Quality Systems/GLP Review:

Print Signature Date

Modification (IACUC APPROVED) / GLP REVIEWED Date: 7 July 2015

USAPHC IACUC Animal Use Protocol Modification

Study Director/ Principal Investigator: **Allison Jackovitz**

PROTOCOL #: **80-14-07-02** Protocol Approval Date: 03 July 2014

PROTOCOL TITLE: **One-generation reproductive toxicity test in Japanese quail (Coturnix japonica) using 3-nitro-1,2,4-triazol-5-one (NTO)**

Phone No.: 410-436-8772 FAX No.:

Email: allison.m.jackovitz.civ@mail.mil

Modification Request #: 6 Submission Date: 2 July 2015

1. Brief non-technical synopsis of existing protocol or background information.

NTO is being investigated as a less sensitive replacement for traditional explosives such as TNT and RDX. NTO must not only meet certain performance criteria, but must also be acceptable from the perspective of human health and the environment. Prior data suggests that NTO may cause problems in the endocrine system, therefore; this study will assess the reproductive and developmental toxicity of NTO using a test in Japanese quail. First, a group of birds were used to determine the doses for the one-generation study. Then, eggs were set and incubated, and birds that hatched (parental/F0 generation) were grown and given various doses of NTO orally. The effects on their body weight and behavior, as well as effects on reproductive and immunological parameters, were evaluated. Eggs were set and incubated from F0 birds, and the birds that hatched (F1) will be evaluated similarly.

2. Type(s) of Modification Requested: (check all that apply)

	Type Of Change	X
ADMINISTRATIVE – AURO/QAU/SEMO REVIEW	Administrative Modifications (*e.g., correct typographical errors/grammar, contact information update, change in study start/completion dates, change in references, protocol title change, change that requires review and approval for GLP compliance*)	
	Addition or deletion of a qualified technician, co-investigator, or study staff	
MINOR Modifications *(examples)*	Change in animal usage [*e.g., vendor, sex, age, weight, strain, small increase in # of animals used (less than 10% of overall number of approved animals)*]	
	Need to repeat an experiment without the addition of animals	
	Addition/ change of sample collection times	
	Additional noninvasive sampling	
	Changes in acclimation or recovery period	
	Special housing request or change in husbandry procedures	
	Changes in dosing procedures (e.g., dose, volume, or timing)	
	Other: _____	X

VETERINARY VERIFICATION AND CONSULTATION (VVC)	Anesthetic, analgesic, sedation changes, or experimental substances (*addition of, change of, or withholding of planned use of*) (e.g.of experimental substances: vehicles, controls; not test articles)	
	Change in euthanasia method to another any approved AVMA Guidelines method (*method only, not change in study endpoint*)	
	Changes in the duration, frequency, type, or number of procedures performed on an animal	
MAJOR Modifications *(examples)*	Change in SD/PI	
	Change in objectives of the study	
	Addition of a test article to be evaluated	
	Change that results in greater pain, distress, or degree of invasiveness (changes to pain categories D or E from C require a literature search for alternatives to be completed)	
	Addition of animals to pain Category E (literature search for alternatives required)	
	Addition of or change in Species	
	Addition of more than 10% of the total # of animals originally approved	
	Addition of blood sampling	
	Change in frequency of observations for morbidity	
	Change in study endpoint	
	Changes in housing or us of animals in a location that is not part of the animal program overseen by the IACUC	
	Changes from nonsurvival to survival surgery	
	Change that impacts personnel safety	
	Other change that relates to the specific experimental design and aims of the original protocol	

3. PREVIOUSLY APPROVED MODIFICATIONS: (*Hit 'Enter' after description of each modification to list all individually.*)

Mod #	Short Description of the Amendment(s) (Include pain category breakdown of animal usage if changes were made from the original protocol)	No. & Species of Animal Requested	Approval Date
1	Incubator should be between 99.0 and 100.0 degrees Farenheit. Addition/removal of study personnel.	N/A	18 Dec 2014
2	Amendment of Table 2. Increase in the number of sentinels. Wider blood draw window. Adendment of Pain Categories. Addition of control recovery	N/A	03 Mar 2015

Mod #	Short Description of the Amendment(s) (Include pain category breakdown of animal usage if changes were made from the original protocol)	No. & Species of Animal Requested	Approval Date
	group.		
3	Addition of 12 recovery birds to the medium high (500 mg/kg) dose group.	N/A	26 Mar 2015
4	Removal of Matthew Bazar, Marc Williams, and Emily Reinke from Study Staff. Due to fewer dose groups and thus animals, workload is reduced.	N/A	28 May 2015
5	Early euthanasia of control recovery animals.	N/A	10 Jun 2015

4. CURRENT APPROVED ANIMAL USAGE ON PROTOCOL:

Species/Total: 584 **B:** 6 **C:** 554 **D:** 0 **E:** 24

5. FOR ITEMS A-G BELOW: Describe the requested changes to be implemented and the justification(s) for each.

A. Specific changes or additions to the experimental design of the protocol (Section V.1. Experimental Design).

As many as 18 males and 18 females from the F1 generation from each dose group (3 dose groups) will be kept for exposure to NTO instead of the 16 per sex per dose that were outlined in Mod #2. The current protocol states the number of birds to continue to be dosed at 4-5 weeks of age would be reduced to 16 per sex per dose group upon moving into adult caging. Then the number would be reduced further to 12 per sex per dose group at 5+ weeks of age in order to establish the breeding pairs. All the birds have been receiving daily doses of the test article orally since day 2 of age. The number of birds will not be culled as stated previously in mod #2 in order to ensure adequate numbers of breeding pairs to continue through the duration of the protocol until termination (when the birds are at approximately 10 weeks of age).

Early in the F0 generation, the 1000 mg/kg dose group was eliminated, due to higher toxicity than expected in an avian model. Later in the generation, the 500 mg/kg dose group was also eliminated. Therefore, only F0 birds from three dose groups were mated and only F1 birds from three dose groups were produced. For the F1 generation, NTO exposure began in ovo via maternal deposition.

At day 2 of age, all birds in theF1 began receiving NTO orally. All hatched birds were exposed since sex cannot be determined early on, and an even number of males are females are necessary to produce breeding pairs. Since all F1 birds have been exposed embryonically and dosed since day 2, keeping as many as 18 males and 18 females from each treatment group does not increase the number of animals used or their pain categories (it means that the birds are not culled from the study). Prolonging exposure by roughly 6 weeks will, however, ensure stronger statistics for the remaining dose groups (if a member from a pair dies, that breeding pair has to be excluded from the data).

B. Change in sample size evaluation, data analysis plan, archiving of data, (Section V.2.).

N/A

C. Change in the animals used, (Section V.3). *(Include any changes in amount used, sex, age/weight, vendor/source, refinement, reduction, and replacement [3Rs]).*

See 5.A.

D. Changes to technical methods (Section V.4.). *(Include changes in USDA pain category classifications [section V.4.1.1.1.1 thru V.4.1.1.1.4.], changes in anesthesia, analgesia, restraint, injections, identification, behavior studies, 'other procedures', study endpoint, euthanasia, etc. Include reference source for basis of dose/treatment change.).*
*** * * * If the modification requested will be adding animals to COLUMN D or E and the protocol did not previously have animals in those categories, a LITERATURE SEARCH FOR ALTERNATIVES TO PAINFUL OR DISTRESSFUL PROCEDURES must be completed and submitted with this modification request.**

N/A

E. Changes to the Husbandry and Veterinary Care procedures (Section V.5.). *(Changes in husbandry considerations, special provisions, exceptions to the Guide, AWAR, or IACUC Policy that have an impact on animal care and us; any changes to the veterinary medical care or environmental enrichment.)*

N/A

F. Changes to the personnel conducting this protocol (Section VI.). *(Include record of completed training for any animal handling or use procedures assigned to new personnel. Signed assurance page(s) must be included with the modification if changing SD/PI, Primary Co-investigoator, or adding a co-investigator.)*

N/A

G. Changes in Biohazard/ Safety (Section VII).

N/A